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Annual Report 2014

Department of Medicinal Chemistry

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Front page illustration: Dr Francesco Russo

Introduction

2014 has been a successful year for the department, thanks to the dedication of our staff. I wish to thank the PhD students, lecturers, researchers, professors and administrative personal for their engagement in research, education, external collaborations, and administrative support.

Research
Research at the Department of Medicinal Chemistry is organised in four divisions: Analytical Pharmaceutical Chemistry, Pharmacognosy, Organic Pharmaceutical Chemistry and the Preclinical PET Platform. Research is conducted in ten groups:

- *Development of analytical methodology for studies in metabolomics*
- *Drug metabolism and drugs in the environment*
- *Molecular pharmacognosy – exploring phylogeny and chemography*
- *Peptide chemical biology*
- *Design and synthesis of new antibiotics and antiviral drug candidates*
- *New peptidomimetics*
- *Non-resonant microwave-heated flow synthesis*
- *Development and mechanistic studies of new synthetic transformation*
- *PET imaging within metabolic diseases, neuroscience and oncology*
- *Development of new PET tracers.*

In 2014 the department secured grants from various funding sources, e.g. the Swedish Research Council, Knut and Alice Wallenberg Foundation, VINNOVA, the Swedish Cancer Institute, and the Swedish Childhood Cancer Foundation. One research area attracting major funding is new antibiotics and antiviral drugs.

The Innovative Medicines Initiative (IMI), with support from the European Commission and major pharmaceutical companies (through EFPIA, the European Federation of Pharmaceutical Industries and Associations), recently launched the 'New Drugs 4 Bad Bugs (ND4BB)' initiative. This is a series of programmes designed to address the scientific challenges associated with antibacterial drug discovery and development.

Within ND4BB the European Gram-Negative Antibacterial Engine (ENABLE), an antibacterial drug discovery platform, has been set up. The aim is to speed up the flow of new antibacterial drugs by moving antibacterial programmes through preclinical discovery and complete phase 1 clinical trials, and produce at least one novel anti-bacterial for systemic Gram-negative infections by 2019. Professor Anders Karlén is co-coordinator and leader of the managing entity for this EUR 85 million project, which will run between 2014 and 2020. ENABLE has been in operation slightly over a year and is running several antibacterial programmes in parallel.

Prof. Karlén and collegues also received financial support (SEK 13.3 million) from the Swedish Research Council for the RAPID project (An Incubator of Hits for Anti-Infective Drug Discovery Programmes). The aim is to identify novel antimicrobial starting points (hits) that target Gram-negative pathogens, or the causative agents of tuberculosis and malaria.

Exploring ion channel toxins from Swedish fauna is a new project supported by the Swedish Research Council. The overall aim of the project, led by Prof. Ulf Göransson, is to explore the pharmacological potential of a neglected source of animal toxins, the peptide toxins. Marine worms (nemerteans) use these toxins in defence and to capture their prey. Preliminary studies show that these peptide toxins work instantly, and that they probably target ion channels in invertebrates and vertebrates. This multidisciplinary project contains elements of pharmacognosy, peptide/protein chemistry, pharmacology, and structural and molecular biology.

Professor Mats Larhed, together with colleagues at Uppsala University and Karolinska Institute, was awarded a grant of SEK 28 million from the Knut and Alice Wallenberg Foundation to set up the national molecular imaging infrastructure WIPPET (Wallenberg Infrastructure Preclinical PET/MRI). The infrastructure will be based in the Preclinical PET Platform at the Department of Medicinal Chemistry.

In 2014, Professor Larhed was awarded the prestigious Ulla and Stig Holmquist Science Prize in Organic Chemistry for his pioneering work in that discipline.

PhD education
A new, VINNOVA-funded national research school in Drug Discovery and Development was started in December 2014. The target is to prepare 30 PhD students and postdocs for either a future career in, or in collaboration with, the pharmaceutical industry, or as entrepreneurs. The research school is based at Karolinska Institute and Uppsala University, in collaboration with SciLifeLab and industrial partners. The Department of Medicinal Chemistry is responsible for the school at Uppsala University.

In 2014, several research groups initiated PhD projects in collaboration with international research groups. Kuei-Hung Lai joined the Division of Pharmacognosy, as the first PhD student in the dual-degree programme arranged by Uppsala University and Kaohsiung Medical University in Taiwan. Astrid Henz, MSc, was recruited as a PhD student at the Division of Pharmacognosy in the EU-financed ITN (Initial Training Network) 'MedPlant' under the supervision of Prof. Anders Backlund. Also affiliated to the MedPlant ITS is Dr. Rosa Buonfiglio, recruited as 'experienced researcher' in the computational chemistry group at AstraZeneca R&D in Mölndal, under the supervision of Dr. Thierry Kogej. Together Henz, Buonfiglio and the collaborative network they are building add further momentum to the chemography research, based on ChemGPS-NPS, carried out at the Division of Pharmacognosy.

Undergraduate education
The teachers have worked on a new educational programme for pharmacists, and we are looking forward to implementing the modernised and improved programme. This task must be completed efficiently, as it diverts valuable time and resources from both teaching and research. Workshops and seminars have been successfully revised, with a focus on improving the students' problem-solving skills and understanding, and learning outcomes and examinations have also been revised. Grants awarded to improve quality in teaching have been used for the courses in Medicinal Chemistry and General Chemistry.

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.

We are pleased to note that the Pharmaceutical Student Council´s pedagogic prize in 2014 was awarded to the teachers at the Division of Organic Pharmaceutical Chemistry.

A more detailed description of undergraduate education at the Department of Medicinal Chemistry is given later in this annual report.

External collaborations
As part of the action plan of the Swedish National Pharmaceutical Strategy, the Division of Analytical Pharmaceutical Chemistry looked into using measured concentration levels of pharmaceuticals found in the Swedish environment as an environmental indicator. The work began by exploring whether a baseline could be obtained using previous measurements of pharmaceuticals (59 reports) in the environment. The reports were evaluated against data reliability criteria, using international guidelines. Half of the reports were found to be reliable, representing a wide geographical spread across Sweden, and covering approximately 100 different pharmaceuticals. The data set produced could be used to form a baseline with which to compare future measurements. The study was presented in October 2014 and the final report issued in January 2015.

Mats Larhed has been project coordinator for EIT Health at Uppsala University since 2012. He is the Scandinavian Board member of the EIT Health European Executive Committee and was recently nominated as Uppsala University's representative on the Board of Directors of the Scandinavian EIT Health Association.

The SciLifeLab Drug Discovery and Development Medicinal Chemistry Lead Identification facility started at the Department in 2014. The facility takes on, explores, and optimises lead-series resulting from hit-2-lead projects. Synthetic and computational chemistry will be at the core of the Design-Make-Test-Analyse

cycle that characterises a small molecule drug discovery programme. The mission is to deliver small drug-like molecules with potency, selectivity, physicochemical, and ADMET properties of sufficient quality to allow proof-of-concept animal studies.

Finance
Basic funding for most of our activities is provided by the government, and the amount has been relatively stable in recent years. I am pleased to note that our research has successfully attracted 50% more external grants in 2014 compared to 2013. The department has recruited several new members of staff, increasing personnel costs by 13%. We must become even more successful in attracting external funding and reducing our costs to attain financial stability. In 2014 we began an overview of our premises – these need to be made more appropriate to our research activities, and costs must be reduced.

The major revenue and expenditure items of the department are as follows.

Major revenues 2014 (SEK million)	
Research and graduate education (public funding)	24 650
Research grants	19 027
Research commissioned	2 768
Education – basic and advanced level (public funding)	22 832

Major Expenditures 2014 (SEK million)	
Personnel costs	39 037
Operating expenses	8 236
Premises	14 935
University/faculty support activities incl. library	7 752
Depreciation	3 234
Travel	1 320

On behalf of the Department, I welcome our new staff and students, and look forward to our collaboration in moving our Department forward. I would also like to take this opportunity to thank those who left our department during 2014 for their excellent contributions over the years.

In 2014 Professor Bo Öberg was appointed Honorary Doctor at the Faculty of Pharmacy. He is a highly respected researcher and an accomplished virologist. Professor Öberg has been involved in research at the Department of Medicinal Chemistry for many years, and played a key role in starting the projects that later led to the discovery of pharmaceuticals for treating patients with HIV and HCV viruses. The HCV project was performed in collaboration with researchers at Stockholm University, Linköping University, Uppsala University and the pharmaceutical company Medivir. As a result of this collaboration, Medivir developed Simeprevir, which so far has been successfully used to treat 70,000 HCV patients.

In memoriam, we remember J Lars G Nilsson who passed away at the age of 76. In 1976, he was appointed adjunct professor in medicinal chemistry, and was a highly respected and popular member of the Department of Organic Pharmaceutical Chemistry, which later became part of the Department of Medicinal Chemistry. His research group comprised Uli Hacksell, Lars-Erik Arvidsson, Uno Svensson and Anette Johansson. The group enjoyed successful collaboration with Professor Arvid Carlsson at the University of Gothenburg, with research focusing on rigid dopamine analogues.

This Annual Review presents a brief summary of the activities of the Department of Medicinal Chemistry during 2014. More information can be found on our web sites (www.farmfak.uu.se/analyt; www.orgfarm.uu.se; fkogserver.bmc.uu.se and pet.medchem.uu.se). Please do not hesitate to contact us personally if you wish to find out more.

Curt Pettersson, PhD, Professor
Head of Department of Medicinal Chemistry
Uppsala University

List of Contents

Organization

Department of Medicinal Chemistry

Head of Department
Curt Pettersson

Deputy Head of Department
Anders Karlén

Department board
Curt Pettersson, chairman
Gunilla Eriksson, technical/administrative and secretary
Ulf Göransson, teacher representative
Mats Larhed, teacher representative
Anja Sandström, teacher representative
Jonas Rydfjord, graduate student representative
Albert Elmsjö, graduate student representative
Martin Svahn, undergraduate student representative
Ylva Hedeland, teacher representative, deputy
Anders Backlund, teacher representative, deputy
Sorin Srbu, technical/administrative
Stefan Svahn, graduate student representative
Rebecka Isaksson, graduate student representative, deputy
Camilo Persson, graduate student representative, deputy

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

Senior lecturer emeriti
Uno Svensson

Director of graduate studies
Anders Backlund

Secretariat
Gunilla Eriksson
Anna-Helena Brandhammar
Olof Jonsson

Course Administration
Maj Blad
Birgitta Hellsing

Computers/IT
Anders Backlund
Sergio Estrada/Olof Eriksson
Jakob Haglöf/Axel Rydevik
Anders Karlén
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
Vt2014
Anders Karlén (50%)
Anja Sandström (50%)
Ht 2014
Ulrika Rosenström(50%)
Christian Sköld (50%

Preklinical PET Platform
Head of Platform
Mats Larhed

Pharmacognosy
Head of Division
Lars Bohlin

Director of undergraduate studies
Anders Backlund

Assignments of staff members

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

Gunnar Antoni
- 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
- Member of the International Editorial Reviewer Board of International Journal of Diagnostic Imaging (IJDI)

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
- Honorary visiting professor at Kaohsiung Medical University
- Director of graduate studies at the Faculty of Pharmacy, Uppsala University
- Director of undergraduate studies in pharmacognosy
- Member of the ULLA ExCo
- Member of the board of Uppsala University Center for Sustainable Development
- Member of evaluation committé at Rannís - the Icelandic Research Council
- Member of the MedPlant ITN supervisory board
- Faculty opponent at Åbo Akademi 2013
- 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)

Lars Bohlin
- Head of division of Pharmacognosy
- Evaluation expert for research projects, Australian Research Council
- Member of the American Society of Pharmacognosy
- Member of the American Botanical Council
- Member of the Editorial Advisory Board of Journal of Natural Products, USA
- Chairman of the Board at Folkuniversitetet, Uppsala
- Member of the Swedish Academy of Pharmaceutical

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

Ulf Göransson
- Associate Editor "Phytochemistry"
- Member of the Editorial Advisory Board "Peptidomics"
- Member of the International Council, Uppsala University
- Deputy 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

Jakob Haglöf
- 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

Anders Hallberg
- Member of the Royal Society of Sciences in Uppsala
- 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
- 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

Anders Karlén
- Vice chairman of the Committee for undergraduate studies (GRUFF), Faculty of Pharmacy, Uppsala University
- 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
- Head of the Division of Organic Pharmaceutical Chemistry
- Head of the Preclinical PET platform
- Deputy Vice President, Medicine and Pharmacy, Uppsala University
- Member of the Pharmaceutical Faculty Committee
- Member of the Swedish Academy of Pharmaceutical Sciences
- Member of the American Chemical Society

- Member of InnoLIFE Executive Committee
- Member of the Editorial Board for ChemistryOPEN
- Member of the Royal Society of Sciences at Uppsala
- Director, SciLifeLab DDD, Medicinal Chemistry - Lead Identification
- Chair of the steering group for the Drug Discovery and Development collaboration

Luke Odell
- Member of The Swedish Academy of Pharmaceutical Sciences
- Member of the Swedish Chemical Society
- Director of Studies for the Drug Discovery and Development – Competence and Resource Network
- Member of the Editorial Board for Current Microwave Chemistry

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 the editorial board of International Journal of Organic Chemistry
- Member of the editorial board of Scientifica
- Member of the editorial board of BioMed Research International
- Member of the Technical Advisory Board of Affibody AB, Solna
- Responsible for animal experiments in ROS, Medical Faculty (Djurföreståndare)
- Assistant (Suppleant) in Jordbruksverkets nationella kommitté from Uppsala University
- Assistant (Suppleant) in Animal etics commeettee 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
- Member of the Section for Pharmaceutical and Biomedical Analysis, at the Swedish Academy of Pharmaceutical Science

Anja Sandström
- Member of the Committee for undergraduate studies (GRUFF), Faculty of Pharmacy, Uppsala University
- Director of undergraduate studies in organic pharmaceutical chemistry
- Chairman of the student recruitment group (STURE), Faculty of Pharmacy, Uppsala University
- Member of the Pharmaceutical Faculty Committee
- Member of the Swedish Academy of Pharmaceutical Sciences
- Member of the American Chemical Society
- Member of the Editorial Board of Frontiers in Chemical Biology

Christian Sköld
- Pharmaceutical profile coordinator for the Master programme in Chemical Engineering, Faculty of Science and Technology, Uppsala University
- Member of the Program committee for the Master programme in Chemical Engineering, Faculty of Science and Technology, Uppsala University
- 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

Ulrika Rosenström
- Director of undergraduate studies in organic pharmaceutical chemistry
- Member of the Committee for equality, Disciplinary Domain of Medicine and Pharmacy, Uppsala University
- Member of The Swedish Academy of Pharmaceutical Sciences
- Member of the Swedish Chemical Society

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. During 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 the 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
- Chiral separation methods
- Capillary electrophoresis for biomedical applications

Members of the group during 2014

Curt Pettersson, Professor
Torbjörn Arvidsson, Associate Professor
Ahmad Amini, Assoicate Professor
Albert Elmsjö, PhD, Researcher
Mikael Engskog, PhD, Researcher
Olle Gyllenhaal, Associate Professor
Mikael Hedeland, Visiting Professor
Ylva Hedeland, PhD, Senior Lecturer
Monika Johansson, Associate Professor
Lars B Nilsson, PhD, Researcher
Niklas Tyrefors, PhD, Researcher
Victoria Barclay, PhD student
Jakob Haglöf, PhD, Junior Lecturer
Cari Sänger-van de Griend
Alexander Hellqvist, PhD student

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

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

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 that the need for robust, validated and accurate analytical methodology starting from design of experiments through 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. In collaboration with the Department of Medical Sciences (UU), the division is focusing on fast screening experiments of various pharmaceutical active substances and their effect on colon cancer cells (Mats Gustafsson and Ulf Hammerling). Moreover, 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) as well as the metabolic consequences of hearing loss caused by cisplatin treatment in patients (UU, Department of Surgical Sciences, Göran Laurell and Pernilla Videhult Pierre). In collaboration with assistant professor 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 neurotoxoicology in collaboration with Professor Eva Brittebo (UU, Department of Pharmaceutical Biosciences), nutrition-based metabolomics (Ulf Risérus, Department of Public Health and Caring Sciences) and Graft-vs-Host disease (Professor Moustapha Hassan, KI, Department of Laboratory Medicine).

Publications 2012-2014

1. Engskog MK, Karlsson O, Haglöf J, Elmsjö A, Brittebo E, Arvidsson T, Pettersson C. The cyanobacterial amino acid β-N-methylamino-l-alanine perturbs the intermediary metabolism in neonatal rats. Toxicology. 2013 Oct 4;312:6-11
2. 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

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.

The ongoing project is focusing on the degradation of β-blocking agents in Californian wetlands. By comparing different wetland types and conditions, the degradation pathways and rates are being estimated. This work includes metabolite identification and quantification using isotopically labeled standards. Furthermore, chiral separation methods are developed and applied to estimate the enantiomeric fractions. Generally, the complex environmental samples are pretreated with solid-phase extraction and further analysed with UHPLC or SFC coupled to mass spectrometric detection.

Publications 2012-2014

1. V.K.H. Barclay, N.L. Tyrefors, I.M. Johansson, C.E. Petterson: Trace analysis of fluoxetine and its metabolite norfluoxetine. Part 1: Development of a chiral liquid chromatography-tandem mass spectrometry methods for wastewater samples J. Chromatogr A 1218; 2011, 5587-5596

2. Barclay V, Tyrefors N, Johansson M, Pettersson C. Trace analysis of fluoxetine and its metabolite norfluoxetine. Part II: Enantioselective quantification and studies of matrix effects in raw and treated wastewater by solid phase extraction and liquid chromatography-tandem mass spectrometry J. Chromatogr. A 1227; 2012, 105-114

3. Barclay V, Tyrefors N, Johansson M, Pettersson C. Chiral analysis of metoprolol and two of its metabolites, α-hydroxymetoprolol and deaminated metoprolol, in wastewater using liquid chromatography-tandem mass spectrometry J Chromatogr A 1269; 2012, 208-217

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

It is necessary to study the ecotoxicity of the treated waters. By performing quantitative and qualitative MS-analyses of the target compounds and intermediates, there is a possibility to correlate changes in ecotoxicity to altering water compositions.

At present, the bioremediation of the NSAID diclofenac is studied using the white rot fungi species *Trametes versicolor (T.v)*. So far, inoculation procedures, development of UHPLC-Q-TOF and GC-MS methods, immobilization tests using solid carriers and E-flask experiments including diclofenac, *T.v*. and carriers have been performed. The immobilization of fungi-mycelia to solid carriers is essential for a prolonged continuous use of the bioreactor and an enhanced enzyme production.

Chiral separation methods

Curt Pettersson, Ylva Hedeland, Monica Johansson, Niklas Tyrefors, Olle Gyllenhaal

The Division of Analytical Pharmaceutical Chemistry has a long record of research within the field of chiral separation. The research has primarily been focused on fundamental studies of separation systems (i.e. capillary electrophoresis, CE, liquid chromatography, LC and supercritical chromatography, SFC) in order to facilitate reliable and predictable separations. Several new selectors, either small molecules with a rigid structures (acting as chiral counter-ions) or proteins have been introduced. The selector has either been dissolved in the background electrolyte (CE) or the mobile phase (LC, SFC) as a chiral additive or been chemically immobilised on the stationary phase (LC, SFC). The analytes of interest within this project have primarily been pharmacological active drugs as e.g., □-adrenoaceptor blocking agents, adrenergic agonists and local anaesthetics.

The gained knowledge has been applied on e.g., analysis of chiral drugs and its metabolites/degradation products in an aquatic environment (i.e. chiral analysis of samples from waste water treatment plants), metabolism studies of chiral drugs in living organisms (i.e. animals and fungus) as well as enantiomeric purity determination of drugs.

Publications 2012-2014

1. V.K.H. Barclay, N.L. Tyrefors, I.M. Johansson, C.E. Petterson: Trace analysis of fluoxetine and its metabolite norfluoxetine. Part 1: Development of a chiral liquid chromatography-tandem mass spectrometry methods for wastewater samples J. Chromatogr A 1218; 2011, 5587-5596

2. Barclay V, Tyrefors N, Johansson M, Pettersson C. Trace analysis of fluoxetine and its metabolite norfluoxetine. Part II: Enantioselective quantification and studies of matrix effects in raw and treated wastewater by solid phase extraction and liquid chromatography-tandem mass spectrometry J. Chromatogr. A 1227; 2012, 105-114

3. Barclay V, Tyrefors N, Johansson M, Pettersson C. Chiral analysis of metoprolol and two of its metabolites, α-hydroxymetoprolol and deaminated metoprolol, in wastewater using liquid chromatography-tandem mass spectrometry J Chromatogr A 1269; 2012, 208-217

4. Cari Sänger - van de Griend, Ylva Hedeland and Curt Pettersson " Capillary Electrophoresis: an Attractive Technique for Chiral Separations" Chromatography Today (2013) 6, 32-37

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 the Norwegian School of Veterinary Science (Oslo, Norway).

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2. Alexander Hellqvist, Ylva Hedeland, and Curt Pettersson, "Evaluation of electroosmotic markers in aqueous and non-aqueous capillary electrophoresis". Electrophoresis (2013) 34, 3252-3259 DOI: 10.1002/elps.201300305

Dissertations 2012-2014

1. Alexander Hellqvist. "Electro-osmotic markers and clinical application with capillary electrophoresis". Licentiate Thesis 44, Faculty of Pharmacy, Uppsala University (2013)

Virus and Vaccine Characterization with Capillary Electrophoresis

Cari van de Griend, Curt Pettersson, Marta Germano (Crucell), Govert Somsen (Vrije Universiteir Amsterdam), Ewoud van Tricht

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 Crucell Holland in Leiden on the development of CE as a platform for viruses and vaccines is unique and pushes the frontiers for vaccine characterization. During 2014, several lectures and posters were presented by 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.
In summary, the presented 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.

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 implemented as alternative to quantitative polymerase chain reaction (qPCR) and anion exchange chromatography (AEX-HPLC). Adsorption issues, typically seen for LC-based method, were in an extensive study solved for CE. The method was successfully validated and applied to 5 representative samples from the process containing cell debri, 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.

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).

Bioanalysis of drugs and their metabolites, drug metabolite production and identification with mass spectrometry

Research Group Leader: Ulf Bondesson

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 is often undesired as such systems require the use of material of animal or human origin.

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 2014

Ulf Bondesson, Adjunct Professor
Mikael Hedeland, Visiting Professor
Axel Rydevik, PhD student
Annelie Hansson, PhD student
Hanna Thorén, student

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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 that are addressed are viral infections caused by HIV and HCV (Hepatitis C Virus), a number of highly resistant bacterial strains, as well as the infectious diseases malaria and tuberculosis and gram-negative infections. 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

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, Fredrik Svensson, Christian Sköld, Jonas Sävmarker, Puspesh Upadhyay, Prajakta Naik

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 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 receptor 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, Anders Karlén, Mats Larhed, Gunnar Lindeberg, Christian Sköld, Charlotta Wallinder, Vivek Konda, Luke Odell, Jonas Rydfjord, Jonas Sävmarker, Rebecka Isaksson

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 2014

Anders Hallberg, Professor
Anders Karlén, Professor
Mats Larhed, Professor
Luke Odell, Assoc. Professor
Gunnar Lindeberg, Researcher
Christian Sköld, Assoc. Professor
Marc Stevens, PhD student
Karin Engen, PhD student
Vivek Konda, PostDoc
Charlotta Wallinder, Researcher
Ulrika Rosenström, Researcher
Fredrik Svensson, PhD student
Puspesh Upadhyay, PostDoc
Prajakta Naik, PostDoc

Publications 2012-2014

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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, Linda Axelsson, Hitesh Motwani, Maria De Rosa

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

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, Patrik Nordeman, Ashkan Fardost, Linda Åkerbladh, Hitesh Motwani

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, Patrik Nordeman, Ashkan Fardost, Jonas Rydfjord, 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 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.
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.

Green Palladium(II) Catalysis

Mats Larhed, Jonas Sävmarker, Christian Sköld, Jonas Rydfjord, Fredrik Svensson

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 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.

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Heterocyclic Chemistry

Luke Odell, Marc Stevens, Shiao Chow, Rajiv Sawant

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 palladium-catalyzed reactions

Christian Sköld, Fredrik Svensson

Background: After identifying a suitable chemical starting point for a target that compound will serve as reference for the synthesis of structurally similar analogues. 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 Pd-catalyzed reaction mechanisms by means of density functional theory calculations.

Method: We are currently focusing our investigations on Pd(II)-catalyzed reactions and we utilize DFT to calculate the potential energy surface of the reactions. By comparing the energy requirement of competing reaction pathways and effects from employed Pd ligands and solvents valuable information on the reaction system is obtained.

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J. Org. Chem. 79, 2014, 12018–12032

Anti-tuberculosis 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.

Members of the group during 2014

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Publications 2012-2014

1. A. Nordqvist, M. T. Nilsson, O. Lagerlund, D. Muthas, J. Gising, S. Yahiaoui, L. R. Odell, B. R. Srinivasa, M. Larhed, S. L. Mowbray, A. Karlén:
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Design and synthesis of *Mtb* Glutamine Synthetase inhibitors

Anders Karlén, Mats Larhed, Johan Gising, Martin Lindh, Luke Odell

Glutamine synthetase (GS) catalyses the synthesis of glutamine from glutamate and ammonia with concurrent hydrolysis of adenosine triphosphate (ATP). The reaction passes through a phosphorylated tetrahedral intermediate. GS is important in bacterial nitrogen metabolism and the synthesized L-glutamine is also a major component of the cell wall of pathogenic mycobacteria. The potential of *Mtb* GS as a drug target has been established in various studies.

Most reported GS inhibitors mimic the glutamate/glutamine transition state structure and bind in the amino acid binding site of GS. By undertaking a literature survey, virtual screening and synthesis of a small compound library a series of inhibitors of *Mtb* GS have been identified. The alternative binding site in GS that can be targeted is the nucleotide or ATP binding site. Recently, in a high throughput screen (HTS) several novel classes of GS inhibitors were identified and anticipated to bind in the ATP binding site. We have selected two of these classes for further studies and based on X-ray structures derived within RAPID started design and synthesis of GS inhibitors. Based on one of these classes, the imidazopyridines, the SAR has been explored thoroughly and low-micromolar potent inhibitors have been identified. Co-crystallization studies on one of the most potent inhibitors have given insights into the binding mode of this structural class. In the other structural class we could quickly modify our inhibitors to submicromolar potency based on the X-ray structure solved for one of our compounds.

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.

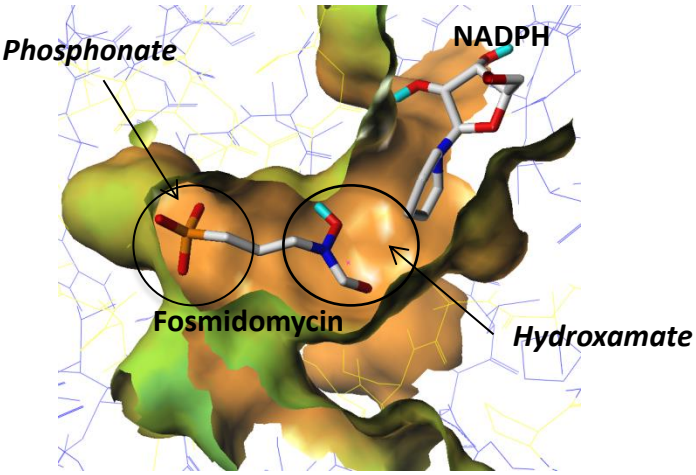

Figure 1. Crystal structure of fosmidomycin bound to *Mtb* DXR. Only the active site is shown for clarity and only part of the NADPH molecule is shown.

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 *Mtb* protease inhibitors

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

We have initiated a project to investigate the possibility that proteases may be useful antituberculosis drug targets. As a first target we selected the proteasome which is a large, multisubunit protease complex central to the regulation of a large number of vital cellular processes. The *Mtb* proteasome is made up of four stacked rings each consisting of seven copies of α and β-subunits. Based on known X-ray structures of the *Mtb* proteasome we have now initiated virtual screening and structure based ligand design studies.

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 antituberculosis 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 2014

Anders Karlén, Professor
Peter Brandt, Associate Professor
Christian Sköld, Associate Professor
Martin Lindh, PhD Student
Hiba Alogheli, PhD Student
Fredrik Svensson, PhD Student
Torbjörn Lundstedt, Adjunct professor

Publications 2012-2014

1. F. Svensson, A. Karlén, C. Sköld: Virtual Screening Data Fusion Using Both Structure- and Ligand-Based Methods. J. Chem. Inf. Model. 52; 2012, 225-232

Virtual screening and library design

Anders Karlén, Peter Brandt, Martin Lindh, Hiba Alogheli, Fredrik Svensson, Christian Sköld, Torbjörn Lundstedt

Many docking programs are very good at reproducing the bound conformation of a ligand in the active site of the protein. However, the scoring functions of these programs generally perform less well at ranking the binding of the ligands in this site. In a virtual screening experiment the scoring function should separate the binders from non-binders. We are therefore studying different approaches to improve this process. In one study we have evaluated different postprocessing methods of the calculated score to increase the number of true binders in a large set of mostly inactive compounds. We are also investigating whether enrichment can be improved by using pharmacophoric post-filtering of docked poses compared with docking alone.

We have also developed a novel design strategy based on the Hierarchical Design of Experiments (HDoE) method named Focused Hierarchical Design of Experiments (FHDoE). This method combines several design layers and uses focused substitutions to increase the probability of designing active compounds when preparing libraries through biasing selection towards a lead structure. We are now evaluating this method in several of our projects.

1. F. Svensson, A. Karlén, C. Sköld: Virtual Screening Data Fusion Using Both Structure- and Ligand-Based Methods. J. Chem. Inf. Model., 52 (2012) 225-232.

Peptides as starting points in drug discovery – On the 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 the latter 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 bioisosteric scaffold 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).

Members of the group during 2014

Anja Sandström, Associate Professor
Eva Åkerblom, Associate Professor
Anders Karlén, Professor
Gunnar Lindeberg, Research Associate
Rebecca Fransson, PhD
Anna Karin Belfrage, PhD student
Johan Gising, PhD student
Hiba Alogheli, PhD student
Ankur Pandey, Postdoctoral Fellow
Anna Skogh, PhD student
Sanjay Borhade, Postdoctoral Fellow
Prasad Wakchaure, Postdoctoral Fellow

Publications 2012-2014

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4. R. Fransson, C. Sköld, J.M. Kratz, R. Svensson, P. Artursson, F. Nyberg, M. Hallberg, A. Sandström*. Constrained H-Phe-Phe-NH2 Analogues with High Affinity to the Substance P 1–7 Binding Site and with Improved Metabolic Stability and Cell Permeability.
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10. R. Fransson, G. Nordvall, J. Bylund, A. Carlsson-Jonsson, J. M Kratz, R. Svensson, P. Artursson, Mathias Hallberg, A. Sandström*. Exploration and Pharmacokinetic Profiling of Phenylalanine Based Carbamates as Novel Substance P 1-7 Analogues.
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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.

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, Gunnar Lindeberg, Rebecca Fransson, Anna Skogh, Ankur Pandey.

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 SP1-7 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, have been and are currently being 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 2012-2014, unrelated to the projects above

1. F. Sehgelmedble, J. Jansson, C. Ray, S. Rosqvist, S. Gustavsson, L. I. Nilsson, A. Minidis, J. Holenz, D. Rotticci, J. Lundkvist, P. I. Arvidsson*: Sulfonimidamides as Sulfonamides Bioisosteres: Rational Evaluation through Synthetic, in Vitro, and in Vivo Studies with γ-Secretase Inhibitors. ChemMedChem, 7 (2012) 396-399.

2. M. F. Maldonado, F. Sehgelmedble, F. Bjarnemark, M. Svensson, J. Åhman, P. I. Arvidsson*: Synthesis and arylation of unprotected sulfonimidamides. Tetrahedron 68 (2012), 7456-7462. DOI: 10.1016/j.tet.2012.06.072

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7. M. M. Makatini, K. Petzold, C. Nahum Alves, P. I. Arvidsson, B. Honarparval, P. Govender, T. Govender, H. G. Kruger, Y. Sayed, J. Lameira, G. E. M. Maguire, M. E. S. Soliman, "Synthesis, 2D-NMR and Molecular Modelling Studies of Pentacycloundecane lactam-Peptides and Peptoids as Potential HIV-1 Wild type C-SA Protease Inhibitors"
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8. U. Yngve, K. Paulsen, I. Macsari, M. Sundström, E. Santangelo, C. Linde, K. Bogar, F. Lake, Y. Besidski, J. Malmborg, K. Strömberg, P. Appelkvist, A. –C. Radesäter, F. Olsson, D. Bergström, R. Klintenberg, P. I. Arvidsson, "Triazolopyrimidinones as γ-Secretase Modulators: Structure-Activity Relationship, Modulator Profile, and in vivo Profiling"
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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 2014, 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 2015.

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 2014.

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 2014

Gunnar Antoni, Associate Professor
Veronika Asplund, Research Engineer
Marie Berglund, PhD student
Sara Bergman, PhD student
Jonas Eriksson, Scientist
Olof Eriksson, Researcher
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, Guest Lecturer
Maria Rosestedt, PhD student
Ramkumar Selvaraju, PhD student
Marc Stevens, PhD student
Alf Thibblin, Assoc. Prof.
Zohreh Varasteh, PhD student
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 2014

Olof Eriksson, Researcher
Ramkumar Selvaraju, PhD student
Marie Berglund, PhD student
Irina Velikyan, Associate Professor
Ewa Hellström-Lindahl, Associate Professor
Jonas Eriksson, Scientist
Ulrika Rosenström, Guest Lecturer

Publications 2012-2014

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Agencies that support the work/Funding

JDRF
Barndiabetesfonden
Diabetesfonden
Diabetes Wellness

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 2014

Mats Larhed, Professor
Gunnar Antoni, Associate Professor
Sergio Estrada, Scientist
Luke Odell, Research Associate
Marc Stevens, PhD student
Charlotta Wallinder, Research Associate

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

Research Group Leader: Gunnar Antoni

Each year many individuals require cranio-maxillofacial surgery as a result of severe injuries, cancer, or birth defects. In the US and Western Europe about 100 000 people are diagnosed with cancer of the head and neck yearly. Traffic accidents, which are expected to rank third in the healthcare burden worldwide by the year 2020, are a major cause of severe injuries with face and head trauma for 50-75% of the accident survivors. Of 10 000 live births, 4-5 infants are born with severe deformities and another 1-2 with jaw anomalies requiring surgery. The outcome of the treatment has profound impact on the quality of life.

There is ample evidence that through detailed planning and advances in implants and graft technology, surgery time, morbidity, and costs are reduced, and the final outcome is significantly improved. We intend to explore methods for *in vivo* early estimations of the integration of implants and grafts. We propose to develop a method based on PET/CT to be used to study the bone response near the interface to implants. We also intend to follow the biological process of bone induction in situations requiring bone augmentation. Available data and previous experiences in this field are not extensive. With the PET technique it has been shown that angiogenesis and new bone is an early event after bone allografts in revision of total hip arthroplasty and PET turned out to be a sensitive method for evaluating neo-vascularization and bone formation in the graft. Further [^{18}F]fluoride PET is a sensitive and useful method for evaluation of bone metabolism using the radiotracer [^{18}F]fluoride to visualize the viability of bone despite the presence of the covering metal component.

Members of the group during 2014

Gunnar Antoni, Associate Professor
Veronika Asplund, Research Engineer

Autoradiography study of angiogenesis in abdominal aortic aneurysm with [18F]fluciclatide – an αVβ3 integrin ligand

Research Group Leader: Gunnar Antoni and Sergio Estrada

The aetiology and pathophysiology of the degenerative process that characterises the development of abdominal aortic aneurysms (AAA) is still mostly unknown. An increased proteolytic activity involving several proteinases has been demonstrated. Histological studies on aneurysms reveal a chronic inflammation in the aortic wall with large amounts of inflammatory cells: T- and B-lymphocytes as well as macrophages. The integrin $\alpha_V\beta_3$ has been identified immunohistochemically in aneurysms, but to our knowledge has never previously been studied with a radioligand in human aortic tissue. The favourable aspect with radioligands are their *in vivo* imaging possibilities, making the large cohort of patients with small AAAs available for more detailed non-invasive pathophysiological molecular investigations. [^{18}F]fluciclatide is a novel PET tracer developed by GE Healthcare, which targets the integrin $\alpha_V\beta_3$

receptor. We hypothesized that angiogenesis may play an important role in the development of AAA and that it could be studied with the PET tracer [^{18}F]fluciclatide. To investigate this we performed *in vitro* autoradiography-, histological-, and immunohistochemical analysis on aneurysmal and normal aortic tissues. The specimens were investigated *in vitro* with [^{18}F]fluciclatide. Aneurysmal aortic tissue showed higher specific uptake of [^{18}F]fluciclatide than non-aneurysmal aortic tissue, although not significant. The uptake of [^{18}F]fluciclatide corresponded to immunohistochemical staining with the $\alpha_V\beta_3$ integrin-receptor antibody LM609. This study suggests that angiogenesis is associated with inflammatory cell infiltration and may play a role in the pathogenesis of abdominal aortic aneurysms. Further *in vitro* and *in vivo* PET studies are planned with this and other PET ligands.
This project is performed in close collaboration with scientists at Uppsala University Hospital.

Members of the group during 2014

Gunnar Antoni, Associate Professor
Sergio Estrada, Scientist
Veronika Asplund, Research Engineer

Publications 2012-2014

1. Tegler G, Estrada S, Hall H, Wanhainen A, Björck M, Sörensen J, Antoni G
Autoradiography Screening of Potential Positron Emission Tomography Tracers for asymptomatic Abdominal Aortic Aneurysms
Ups J Med Sci 119:229-235 2014

Neurodegeneration and other brain disorders

In vitro studies of central and systemic and Aβ-amyloidosis

Research Group Leader: Sergio Estrada

Amyloidosis is characterized by the abnormal extracellular deposition and accumulation of insoluble fibrillar proteins in organs and tissues. Amyloids are arranged in a β-sheet structure and fibril formation has been identified for close to 30 proteins. Deposited amyloid fibrils may contribute to organ dysfunction. The cardiovascular system is often affected by amyloidosis, as well as organs such as liver, kidney and spleen. Four of the most common amyloid associated diseases are: immunoglobulin light chain amyloidosis (AL), transthyretin amyloidosis (TTR), amyloid protein A amyloidosis (AA) and beta amyloidosis (Aβ). In systemic AL amyloidosis, fibrils are derived from a monoclonal immunoglobulin light chain produced by a plasma cell clone. Transthyretin (TTR) is synthesized in the liver and is the plasma protein found in most types of familial amyloidosis and is also the pathologic protein found in senile systemic amyloidosis. AA amyloidosis is a complication of chronic infections and inflammatory diseases in which there is sustained overproduction of the acute phase protein, serum amyloid protein A, which is mainly expressed by the liver. Deposition of Aβ-amyloid in the brain is one of the central neuropathological hallmarks in AD and is a product of sequential cleavage of the amyloid precursor protein, APP.

Pittsburgh compound B (PIB) is a derivative of the amyloid-binding dye thioflavin-T and has been developed for imaging Aβ deposits in AD brain *in vivo* by PET. In previous studies, a positive correlation has been shown between the *in vivo* retention of [^{11}C]PIB and *postmortem* measures of Aβ and binding of both ^{11}C- and ^{3}H-labelled PIB.

In the present project we have characterized [^{3}H]PIB binding *in vitro* to different tissues involved in systemic amyloidosis in comparison to AD brain. *In vitro* binding studies were conducted using [^{3}H]PIB and tissue homogenates of *postmortem* heart, liver, spleen and kidney from patients with TTR, AL, and AA systemic amyloidosis, as well as brain homogenates from AD patients and healthy control subjects. Saturation and competition experiments were performed to determine binding parameters such as K_d, B_{max} and IC_{50} values.

High-affinity binding of [^{3}H]PIB was observed in all tissues from patients with systemic amyloidosis. The mean value of [^{3}H]PIB binding was highest in patients with TTR followed by AL and AA amyloidosis, although a large variation was found between subjects suffering from the same type of amyloidosis. The levels were comparable with those found in cortical regions of AD brain. In AD brain, both high-and low affinity binding sites to [^{3}H]PIB were observed but much less frequent in tissues from patients with systemic amyloidosis.

Members of the group during 2014

Sergio Estrada, Scientist
Gunnar Antoni, Associate Professor

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 2012-2014

1. Bergman S, Estrada S, Hall H, Rahman R, Blomgren A, Larhed M, Svedberg M, Thibblin A, Wångsell F, Antoni G

2. Synthesis and labeling of a piperazine-based library of 11C-labelled ligands for imaging of the Vesicular Acetylcholine Transporter
J Labelled Compd Radiopharm 57:525-532, 2014

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.

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.

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.

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.

Development of PET tracers for the study of fibrosis

Research Group leader: Gunnar Antoni

Gunnar Antoni, Olof Eriksson, Gunnar Lindeberg, 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.

Publications 2012-2014

1. Velikyan I, Eriksson O, Estrada S, Häggström J, Ljungvall I, Rosenström U, Antoni G Synthesis and preclinical evaluation of 68Ga-labeled collagelin analogues for imaging and quantification of fibrosis Nucl Med Biol (2014), http://dx.doi.org/10.1016/j.nucmedbio.2014.06.001

Pre-clinical and clinical PE-CT in vivo and histomorphometrical investigations of bone response, and bone formation in connection with Titanium implants and bone replacement.

Research Group Leaders: Gunnar Antoni, Jan Michael Hirsch

Gunnar Antoni, Veronika Asplund, Christoffer Riben, Jens Sörensen, Andreas Thor, Jan Michael Hirsch

Each year many individuals require cranio-maxillofacial surgery as a result of severe injuries, cancer, or birth defects. In the US and Western Europe about 100 000 people are diagnosed with cancer of the head and neck yearly. Traffic accidents, which are expected to rank third in the healthcare burden worldwide by the year 2020, are a major cause of severe injuries with face and head trauma for 50-75% of the accident survivors. Of 10 000 live births, 4-5 infants are born with severe deformities and another 1-2 with jaw anomalies requiring surgery. The outcome of the treatment has profound impact on the quality life.

There is ample evidence that through detailed planning and advances in implants and graft technology, surgery time, morbidity, and costs are reduced, and the final outcome is significantly improved. We intend to explore methods for *in vivo* early estimations of the integration of implants and grafts. We propose to develop a method based on Positron Emission Tomography – computed tomography (PET-CT) to be used to study the bone response near the interface to implants. We also intend to follow the biological process of bone induction in situations requiring bone augmentation. Available data and previous experiences in this field are not extensive. With the PET technique it has been shown that angiogenesis and new bone is an early event after bone allografts in revision of total hip arthroplasty and PET turned out to be a sensitive method for evaluating neo-vascularization and bone formation in the graft. Further [^{18}F]fluoride PET is a sensitive and useful method for evaluation of bone metabolism using the radiotracer [^{18}F]fluoride to visualize the viability of bone despite the presence of the covering metal component.

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.

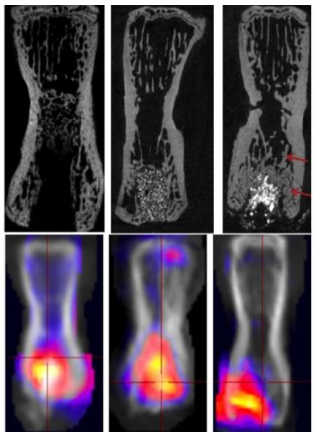

Figure 1: a rat-tail vertebral defect filled with bone substitutes. The lower picture show the osteoblast activity in the defect studied in SPECT with a radioactive tracer.

A new study is planned in collaboration with the platform for preclinical PET using PET and SPECT to follow the kinetics of the bone regeneration process in rats using sodium [125I]iodide, sodium [18F]fluoride.
The kinetics of bone formation is measured both as release of [125I]iodide from the synthetic bone substitutes with SPECT and as osteoblast activity quantified by PET and sodium [18F]fluoride. The angiogenesis process during the re-modeling phase of the bone fillers will be studied using a tracer binding to the $\alpha\nu\beta3$ receptors.

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

Research Group Leaders: Mats Larhed and Gunnar Antoni

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 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 AD 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.

The aim of this project is to design and synthesize selective and stable non-peptidic β-secretase tracers. Furthermore, different strategies for ^{11}C labeling of BACE-1 PET tracers are investigated.

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.

Members of the group during 2014

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

Publications 2012-2014

1. Nordeman P, Estrada S, Odell L, Larhed M, Antoni G
^{11}C-Labeling of a Potent Hydroxyethylamine BACE-1 Inhibitor and Evaluation *in vitro* and *in vivo*
Nuc Med Biol 41:536-543 2014

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

Research Group Leaders: Gunnar Antoni and Håkan Hall

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 thiophene derivatives with ^{11}C and ^{18}F and to 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.

Preliminary autoradiographic studies indicate that two of the thiophene ligands, the ^{11}C-labeled tetrameric compound ([^{11}C]TPHD) and ^{18}F-labeled pentameric compound ([^{18}F]TPHE) bind specifically to amyloid containing brain sections. One single experiment of the binding of one of these ligands ([^{11}C]TPHD) to tissue of a mouse treated to contain amyloidosis in the pancreas. In comparison to the accumulation in brain, the binding to pancreatic amyloidosis was weak, but clearly evident. Hematoxylin-eosin staining of parallel sections verified that the ligands accumulated to amyloidosis of the sections.

Two rat whole-body PET studies were performed with the two promising ligands [^{18}F]TPHE and [^{11}C]TPHF on normal rats of normal age, considered to have no amyloid in the brains. Consequently, very little uptake was found in the brains of these rats. Moreover, PET / CT was performed in a healthy female

Cynomolgus monkey, assumed to have no amyloid depositions in the brain or elsewhere, to study the distribution of the three ligands [^{11}C]TPHB, [^{11}C]TPHD and [^{18}F]TPHE. These in vivo PET studies were performed to see the general distribution of the ligands and to get sufficient pharmacokinetic data before studying animals with amyloidosis, either in brain or systemic.

This project is performed in collaboration with scientists at another department of Uppsala University and with Linköpings University. The project is funded by a three year grant from VINNOVA (2009 – 2012) with similar funding from GE Healthcare and BioArctic Neuroscience AB.

Members of the group during 2014

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

Oncology

Novel radionuclide imaging methods for 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 Biomedical Radiation Sciences and PET Center 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 (170 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. 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 2014

Anna Orlova, Associate Professor
Zohreh Varasteh, PhD student
Maria Rosestedt, , PhD student
Bogdan Mitran, MS student

Development of imaging agents for visualization of GRPRs

1. 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.
2. 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 group during 2014

Anna Orlova, PPP
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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 group during 2014

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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 PDGF1R.
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.

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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 group during 2014

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Thesis defended

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

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Agencies that support the work/Funding

Faculty of Pharmacy	750 kSEK/y, 2013-2014
Vetenskapsrådet	900 kSEK/y, 2013-2015
Cancerfonden	500 kSEK/y, 2013-2014

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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.

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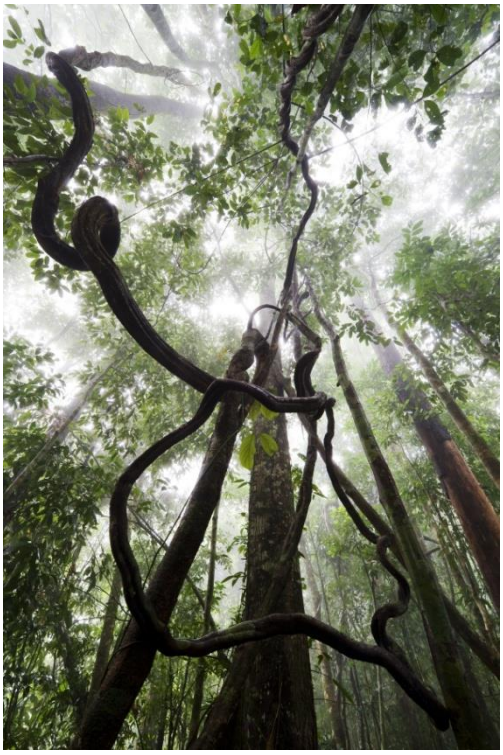
Pharmacognosy

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, systematic botany 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.

The ongoing projects are focused on chemistry and biology of ultra stable proteins, methods of selection and target-finding, antifouling and antibacterial molecules from marine organisms, anti-inflammatory and antitumor activity of natural products.

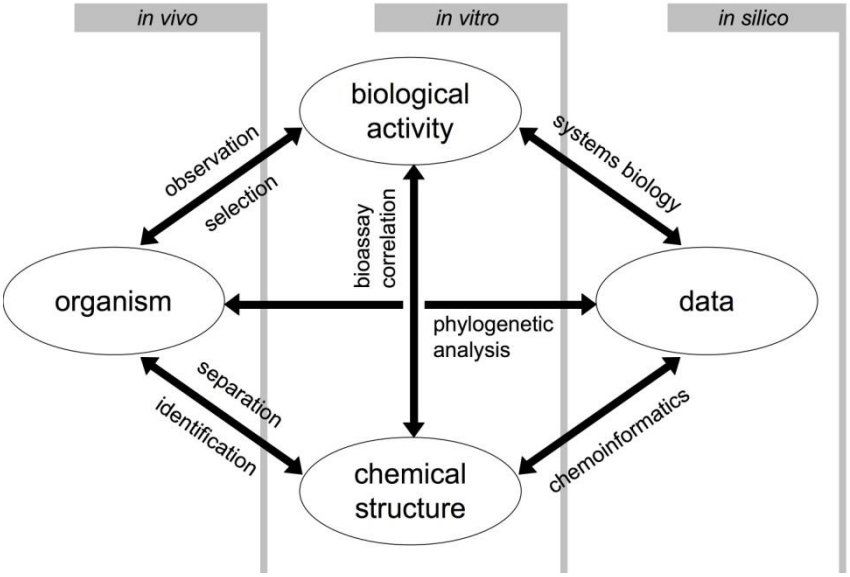


Figure 2. The interdisciplinary nature of pharmacognosy is demonstrated by the explanatory model above (Figure by S. Larsson)

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.

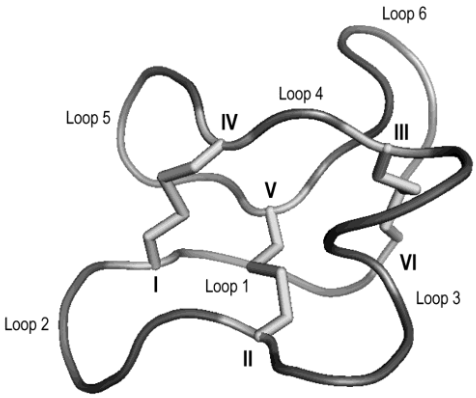


Figure 1. The cyclotide backbone*. Note the circular backbone and the cystine knot that define the cyclic cystine knot (CCK) motif. The variable loop regions (marked 1-6) between the cysteines (marked I-VI) are targets for protein engineering. The CCK motif is able to harness a number of biological activities: 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 two years we have entered into peptide toxin discovery exploiting peptidomics, combining next generation sequencing and mass spectrometry. Some of the research highlights during the year are summarized in Figure 2.

Of particular importance during the first half of the year was the establishment of the open interest group "Uppsala Peptide Chemistry and Biology", which gather researchers with interest in peptide research from three faculties at UU. The first two meetings were held during the fall 2013, and the series of meetings followed during the spring 2014.

Key collaborators during the year 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. We have continued the collaboration with Prof Per-Johan Jakobsson at Karolinska Institutet, about peptides relevant in rheumatoid arthritis.

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 att UU by Prof Lars Bohlin. Dr Paco Cardenas has substantially reinforced our research in that field. Moreover, we collaborate with Dr Håkan Andersson at Linnéuniversitetet, Dr Malin Strand at Göteborgs University, and Dr Per Andren, UU, on marine toxins.

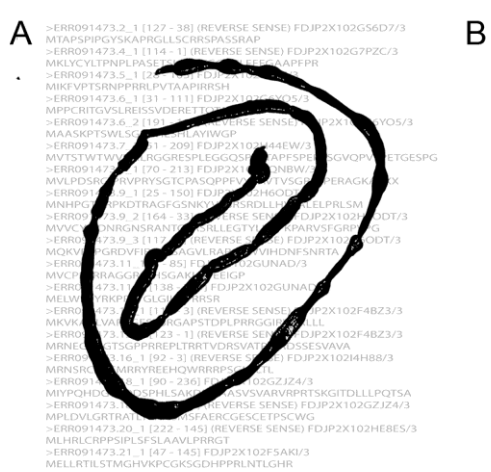


Figure 2. Some research highlights 2014. A) *We now take advantage of the power of next generation sequencing for peptide discovery, here illustrated by the species specific database in the background overlaid by a marine species that has been mapped for peptide toxins.*
B) *This peptide has been isolated from another marine organism, a sponge. We have also solved the first peptide structures in solution in house.*

Our international collaborations include the project of Błażej Ślązak at the Jagiellonian University, Krakow, Poland, and his work of plant cell cultures of endangered Viola species. He obtained his PhD during the fall, under the supervision of Prof Elżbieta Kuta, and Ulf Göransson as assistant supervisor. We have had a continued good collaboration with Drs Johan Rosengren and Richard Clark, and Prof David Craik at the University of Queensland, Australia. Bodil Carstens, who visited the lab during 2013 from the Clark and Craik groups, submitted her thesis under 2014. Again, Ulf was assistant supervisor.

Dr Christian Gruber at the Medical University of Vienna, Prof Lars Skjeldal at The Norwegian University of Life Sciences, and Prof Tatiana Odintsova at the Russian Academy of Sciences should be mentioned among other international collaborators.

We successfully managed to secure a VR Swedish Research Link grant (750 kSEK, 2014-2016) to establish collaboration with University of Colombo, Sri Lanka. Main applicant and the driving force behind this project is Sunithi Gunasekera.

During 2014, students from the Pharmacy Programme, the Uppsala Graduate School for Biomedical Research, and the Summer Research School (SOFOSKO) have been involved in our research. Rebecca Faresjö did her Master research project at UQ in the Clark group.

Members of the group during 2014

Ulf Göransson, PhD, Professor
Sunithi Gunasekera, PhD
Hesham El-Seedi, PhD
Adam Strömstedt, PhD
Delgerbat Boldbaatar, MSc, Guest PhD student, National University of Mongolia
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
Błażej Ślązak, MSc, Guest researcher (Jagiellonian University)
Aida Abd El-Wahed, Guest PhD student, El-Menoufia University
Javid Hussain, MSc, Research Assistant

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2. Yeshak MY, Göransson U, Burman R, Hellman B. (2012) Genotoxicity and Cellular Uptake of Cyclotides: Evidence for Multiple Mode of Action. Mutation Research, Genetic Toxicology and Environmental Mutagenesis 747(2):176-81

3. Yeshak MY, Burman R, Eriksson C, Göransson U. (2012) Optimization of cyclotide extraction parameters. Phytochemistry Letters. 5(4):776-781

4. Svahn KS, Göransson U, El-Seedi H, Bohlin L, Larsson DG, Olsen B, Chryssanthou E. (2012) Antimicrobial activity of filamentous fungi isolated from highly antibiotic-contaminated river sediment. Infectious Ecology and Epidemiology. 2012;2. doi: 10.3402/iee.v2i0.11591.

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1. El-Seedi HR, El-Said AM, Khalifa SA, Göransson U, Bohlin L, Borg-Karlson A-K, Verpoorte R. (2012) Chemistry, natural sources, dietary intake and pharmacokinetic properties of hydroxycinnamic acids. Journal of Agriculture and Food Chemistry. 60(44):10877-95.

2. Göransson U, Burman R, Gunasekera S, Strömstedt AA, Rosengren KJ. (2012) Circular proteins from plants and fungi. Journal of Biological Chemistry. 287(32):27001-27006.

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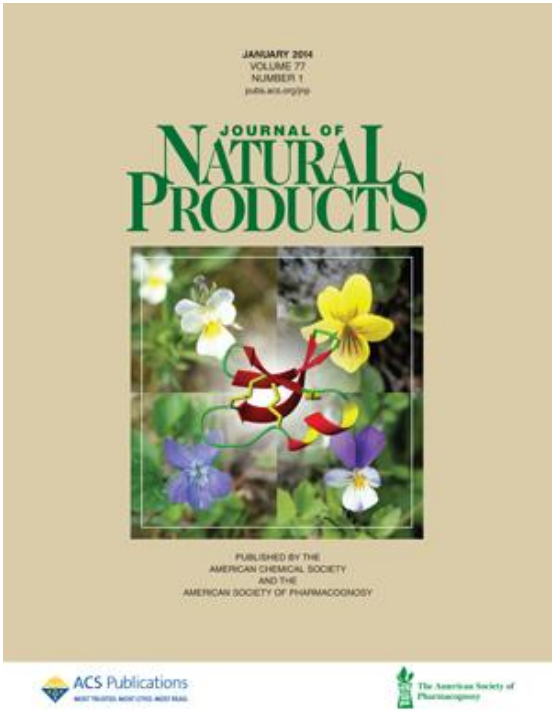

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Agencies that support the work/Funding

Swedish Foundation For Strategic Research, Programme for Future Research Leaders, 850 kSEK
Swedish Research Council, NT, 850 kSEK
Swedish Research Council, MH, 600 kSEK
Scientific Domain of Medicine and Pharmacy UU, 750 kSEK.
Swedish Research Council, Swedish Research Links, 250 kSEK (Sunithi Gunasekera project leader)

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*, *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 million 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 2014

Cecilia Alsmark, Assistant professor
Anders Backlund, Professor
Anna Koptina, PhD., Post doc
Elisabet Vikeved, MSc, PhD student
Åke Strese, MSc, PhD student

Publications 2012-2014

1. Alsmark, C., Strese, Å., Wedén, C., and Backlund, A. Microbial diversity of Alcyonium digitatum. Phytochemistry Reviews. 2012 Jun

2. 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

Reviews 2012-2014

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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 5, below, the web interface is displayed.

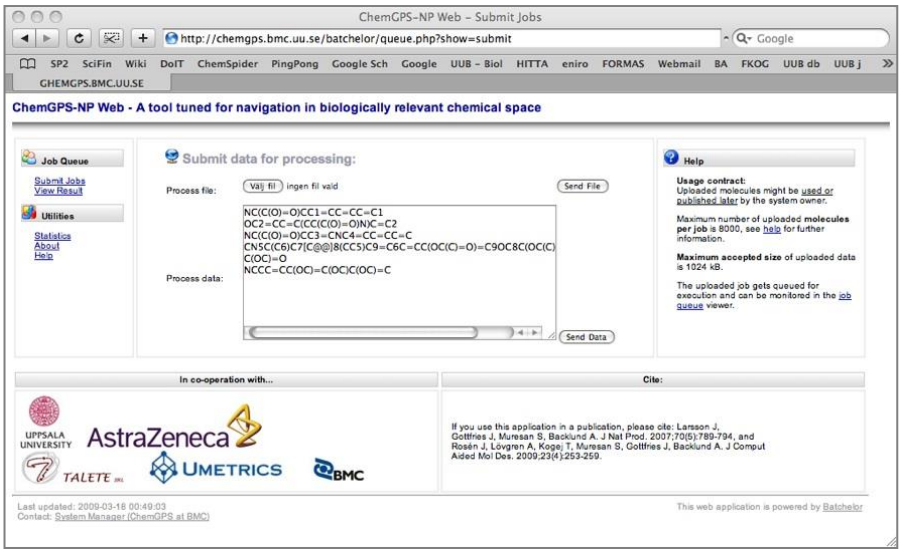

Figure 5. Web interface for ChemGPS-NPweb.

Since the launch in May 2008 more than 9 million compounds originating from more than 6000 users world-wide have been predicted via ChemGPS-NPweb.

Chemographic predictions and Euclidean distances. During 2009 two studies of some significance was published from the group. In the first paper it was shown that a mapping of chemical compounds in ChemGPS-NP provided a prediction regarding the compounds cytotoxic mode of action (MOA) of similar strength to experimental methods previously employed. The developed method was evaluated by comparison with a reference data set from National Cancer Institute (NCI), and provide a significant

improvement. This, in particular, in the sense of making possible predictions of MOA already from chemical structure without necessitating event to have the actual compounds in hand. It must be pointed out, however, that the model does not enable us to predict the actual cytotoxicity, only which MOA that is responsible for an experimental observation of cytotoxicity. Since then the continued development of tools to estimate Euclidean distances, their predictive power in comparison with the frequently utilised Soergel-distance, and the issue of directionality in high-dimensional space been adressed. Several co-operative projects exploring ChemGPS-NP chemical space as a tool for selection of compound libraries and interpretation of results from semi-synthesis and derivatisation of natural products have been initiated with researchers in Taiwan, Finland and Belgium.

In one of these projects, with Frédérick and co-authors, we demonstrate in a study published in *Journal of Medicinal Chemistry*, that chemographic mapping can be used to interpret the cytotoxic activity observed from a series of semi-synthetic derivatives. Based on the physico-chemical properties highlighted from the chemographic mapping, further strategies in compound derivatisation could be suggested.

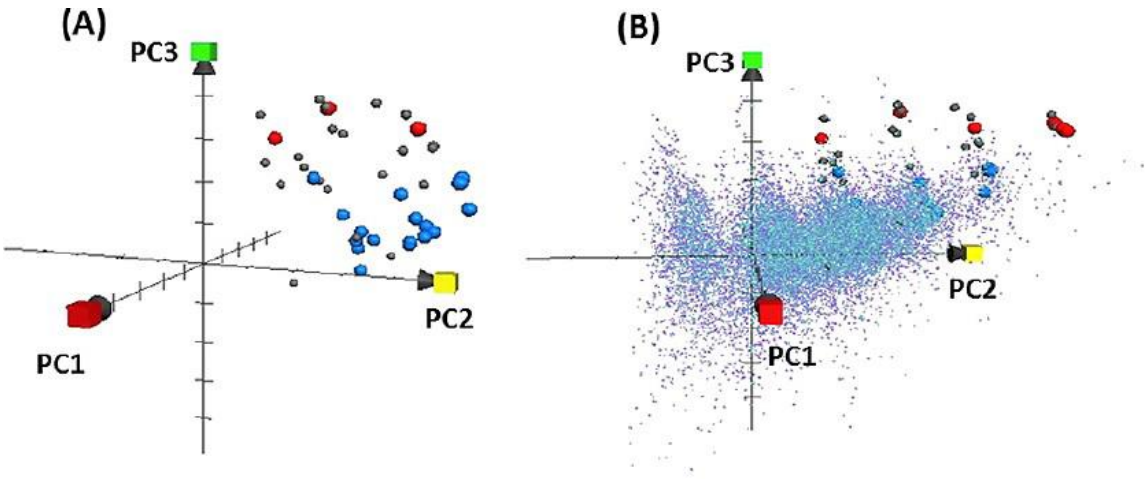

Figure 6. In 6A we can see a set of cytotoxic compounds plotted in chemical property space. Those coloured in red indicates highly potent compounds, those in blue less potent, and the gray compounds with low or intermediate effect. In figure 6B, the tested compounds are related to the ZINC-NP reference set of ca 25 000 compounds, to demonstrate that the highly potent compounds exhibit comparably uncommon properties. From Frédérick *et al.*, 2012 in Journal of Medicinal Chemistry.

A second of these projects resulted in a study combining *in silico* cytotoxicity MOA predictions, with proper biological testing. The purpose being to determine the activities of two novel, cytotoxic, compounds derived from natural sources (Lee et al., 2012). Utilizing our MOA model published by Rosén and co-workers in 2009, combined with Euclidean distance estimates, the two compounds were predicted as inhibitors of the enzyme topoisomerase II (Figure 7). This was subsequently confirmed in the paper from relevant bioassays.

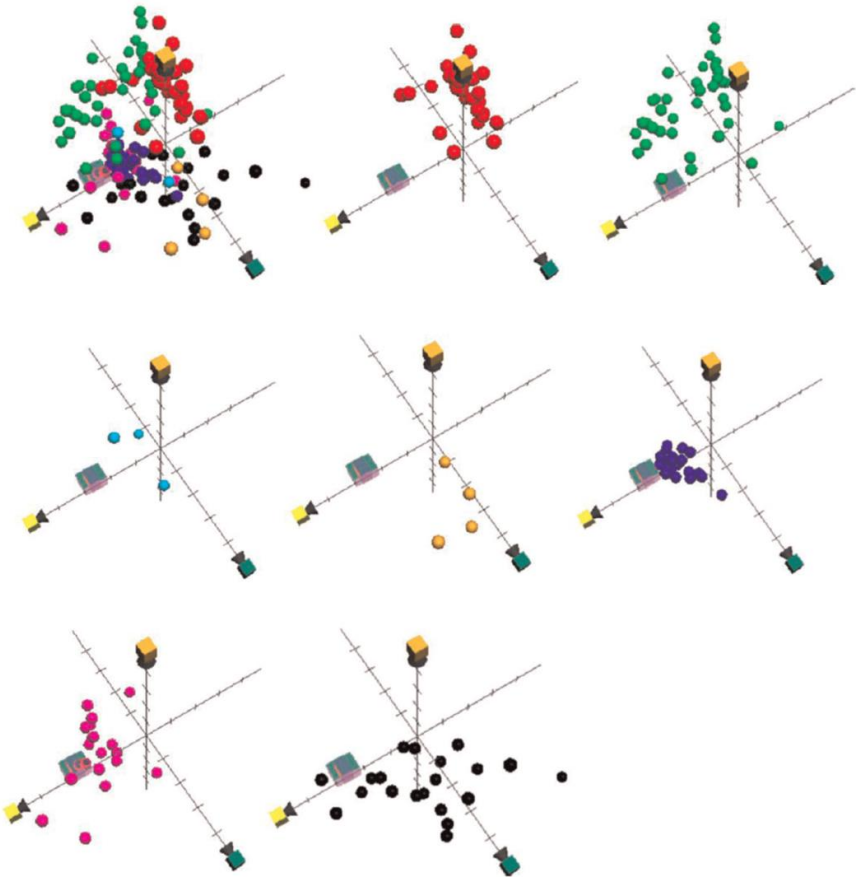

Figure 7. ChemGPS-NP analysis of calanquinone A and denbinobin. Score plot of the three dimensions (principal components 2–4) consisting of PC2 (yellow; aromaticity etc.), PC3 (green; lipophilicity etc.) and PC4 (orange; flexibility/rigidity), from analysis of most potent compounds 6a and 6b as medium seagreen cubes in the ChemGPS-NP model addressed by Rosén et al. in 2009 for prediction of MOA. A reference set of known anticancer agents includes alkylating agents (red), anti-metabolites (lime), proteasome inhibitions (cyan), tyrosine kinase inhibitors (orange), topoisomerase I (blue), topoisomerase II (magenta), and tubulin inhibitors (black). From Lee *et al.,* 2012 in PLoSone.

Connecting phylogenies and chemography. In publications by Catarina Ekenäs and co-workers from 2008 and 2009 the first attempts to correlate bioassay (NF-kB and HNE), chemical (GC-MS and LC-MS), and phylogenetic (DNA sequences) data were made. From the available data it could be shown that on the one hand phylogenetic data and chemical data exhibited significant correlation, even to the extent that putative hybrids and patterns from gene duplications could be traced. During 2012 two additional 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.

Data compiled clearly demonstrates the differences between terrestrial and marine natural products chemistry (Figure 8), as well as both of these to a set of circa 50 000 ‘druglike compounds’ from the Maybridge compound libraries.

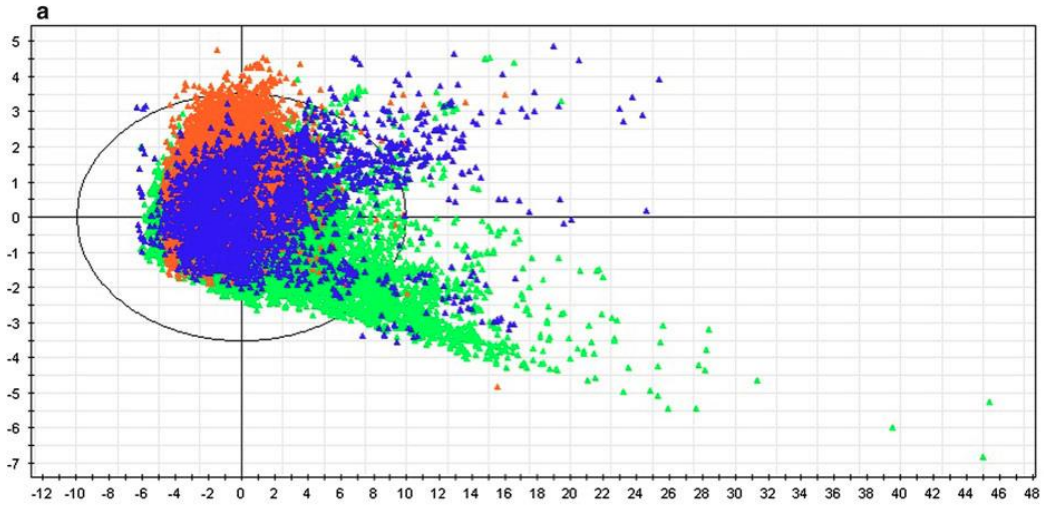

Figure 8. Results from chemographic mapping of marine (blue), terrestrial (green) and druglike (orange) compounds, compiled from literature. Plots clearly demonstrates differential coverage of multi-dimensional chemical property space. From Muigg *et al.*, 2012 in *Phytochemistry Reviews*.

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 9 below.

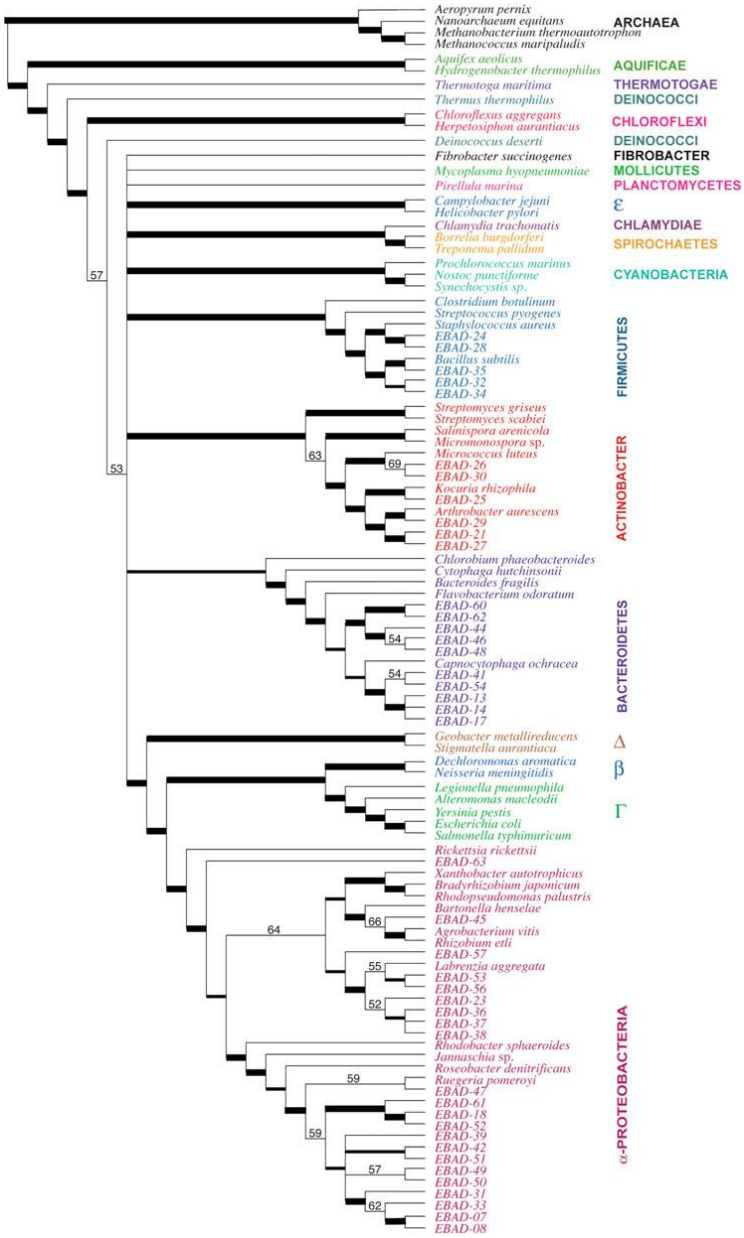

Figure 9. Phylogenetic tree a selected set of reference bacteria, and phylogenetic position of environmental samples from the interior of an *Acyonium* soft-coral (labels EBAD-#). This indicates not only that there is a wide diversity of bacteria living inside the coral, but also that these can be firmly assigned to evolutionary groupings by means of phylogenetic analysis. From Alsmark *et al.*, 2012 in *Phytochemistry Reviews*.

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.

Under 2014 we also welcomed the first PhD-student in the dual-degree program between Uppsala University and Kaohsiung Medical University, MSc. Kuei-Hung Lai, 賴奎宏 working with Prof. Backlund and Dr. Wedén as his supervisors.

Members of the group during 2014

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
Elisabet Vikeved, MSc, PhD student
Astrid Henz, MSc, PhD student
Kuei-Hung Lai, MSc, PhD student
Åke Strese, MSc, PhD student – on paternal leave

Publications 2012-2014

1. Alsmark, C., Strese, Å., Wedén, C., and Backlund, A.: Microbial diversity of *Alcyonium digitatum*. *Phytochemistry Reviews* DOI 10.1007/s11101-012-9229-5 – 2012

2. Lee, C.-L., Lin, Y.-T., Chen, G.-Y., Backlund, A., Yang, J.-C., Wu, C.-C., Hwang, T.-L., Chen, S.-L., Chang, F.-R., and Wu, Y.-C.: Synthesis and biological evaluation of phenanthrene derivatives as cytotoxic, antiplatelet aggregation and anti-inflammatory agents with pharmacophore modeling in the human breast cancer cell line MCF-7 and ChemGPS-NP prediction as topoisomerase II inhibitors. *PLoS ONE* 7: e37897 DOI 10.1371/journal.pone.0037897 – 2012.

3. Frédérick, R., Bruyère, C., Vancraeynest, C., Reniers, J., Meinguet, C., Backlund, A., Masereel, B., Kiss, R., and Wouters, J.: Novel trisubstituted harmine derivatives with original in vitro anticancer activity. *Journal of Medicinal Chemistry* 55, pp 6489-6501 DOI 10.1021/jm300542e – 2012.

4. Muigg, P., Rosén, J., Bohlin, L. and Backlund, A.: In silico comparison of marine, terrestrial and synthetic compounds using ChemGPS-NP for navigating chemical space. *Phytochemistry Reviews* DOI 10.1007/s11101-012-9256-2 – 2013

5. 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.

1.

Reviews 2012-2014

1. Bohlin, L., Alsmark, C., Göransson, U., Klum, M., Wedén, C., and Backlund, A.
Strategies and methods for a sustainable search for bioactive compounds.
in*:* Bioactive Compounds from Natural Sources, ed. C. Tringali.
Taylor & Francis Group / CRC Press, Boca Raton, Fl. – 2012.

Agencies that support the work/Funding during 2014.

European Comission via ITN MedPlant People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° 606895.

Antifouling and antibacterial activity of marine organisms

Research Group Leader: Lars Bohlin

The project is related to the sustainable use of natural products and development of "Green chemistry". The future society needs biodegradable natural products with specific actions and low residence times, e.g. for control of fouling organisms in the marine environment. Marine organisms have shown to contain a wealth of bioactive secondary metabolites with potential for new pharmaceutical or biotechnological applications. Marine sponges produce substances, which have a key role in the defence against pathogens, parasites, predators and biofouling organisms.

In our earlier research we have isolated, characterized and synthesized several cyclopeptides from the marine sponge *Geodia barretti*, with effect on cyprids from *Balanus improvises,* which could explain why this sponge is free from ongrowth of other organisms. The objective of the studies was to further explore the chemical diversity in *Geodia barretti*. Furthermore, the aim was to understand the biological activity on different targets and to evaluate if the compounds produced by the sponge act in concert, either by synergistic or cooperative action, and to investigate a possible bacterial origin of the compounds.

For isolation of minor secondary metabolites state of the art methods for chemical analysis have been used, such as LC-MS, MS/MS and 2D-NMR. For establishing biological activity a barnacle settlement assay *in vitro* has been used to evaluate the effect of the isolated compounds on the behaviour on cyprid larvae. The brominated cyclopeptides have also been tested further for affinity to human serotonin receptors using an *in vitro* radioligand binding assay based on displacement of radioligands from human 5HT-receptors expressed in HEK-293 cell membranes. The cyclopeptides selectively interacted with the serotonin receptors 5-HT_{2A}, 5-HT_{2C} and 5-HT_4 at concentrations close to that of endogenous serotonin.

We here show that the two congeneric defence cyclodipeptides, barettin and 8,9-dihydrobarettin, produced by the coldwater marine sponge *Geodia barretti* act in synergy to deter larvae of surface settlers. An *in situ* sampling using a Remotely Operated Vehicle (ROV) at a depth of 123 m revealed that the sponge continuously releases these two compounds to the ambient water. Previously, we showed that these compounds specifically bind to serotonergic 5-HT receptors. We suggest that the chemical defence in *G. barretti* involves synergistic action, with congeneric compounds produced by the same enzymatic pathway, where one of the targets is a 5-HT receptor and that the synergy of barettin and 8,9-dihydrobarettin have developed to reduce the cost for the sponge to uphold its chemical defence.

Further research has been focused on microfungi and their role in producing secondary metabolites with effect on multi resistant bacteria. Development of methods for cultivation and fermentation of micro fungi is an important part of this project but also detection methods using modern mass spectrometry techniques. Experiments are performed using the in house class 2 laboratory for cultivation of bacteria and antibacterial assays.

Members of the group during 2014

Lars Bohlin, Professor
Ulf Göransson, Professor
Stefan Svahn, PhD student

The marine project is since 2012 involved as a partner in an EU project; From Gene to Bioactive Product. Lars Bohlin, Ulf Göransson, Anders Backlund, Cecilia Alsmark and Anna Koptina.

Anti-inflammatory and anti-tumor activity of natural products

Research Group Leader: Lars Bohlin

The overall aim of our research is to discover substances of natural origin with potential as chemo-preventive agents, or novel leads in the area of inflammation and cancer. Studies on host defence in plants and animals have resulted in discovery of similarities between pathogen recognition, signal transduction pathways and effector mechanisms. This fact, together with scientific reports of the use of many plants to influence diseases of inflammatory origin and cancer, has been the scientific rationale for the project. In our earlier research a number of inhibitors of cycloxygenase-1 and -2 have been discovered, and chemically and pharmacologically characterized using a bioassay guided isolation procedure. In later years the project has developed towards related to anti-tumour activity, especially in colon cancer. A vegetarian diet rich in phytochemicals may prevent colon carcinogenesis by affecting biochemical processes in the colonic mucosa. We have shown that intact faecal water samples from human volunteers significantly decreased prostaglandin production and COX-2 expression in colonic cells. NMR spectroscopy and multivariate data analysis were later used for further analysis of the composition of the faecal waters and to trace the COX-2 inhibiting activity.

The bioactivity of different natural products has been further studied from a chemographic perspective with the aim to understand how to select plants with potential anti-inflammatory activity. A new model ChemGPS-NP has been developed and tested for a series of different datasets, including previously studied COX-2 inhibitors and antitumor substances. The project is now focused on in-depth studies of specific secondary metabolites in plants and their effects on human resistant cancer cell lines, especially colon cancer. The potential synergistic effects of the combination of natural products and conventional cytotoxic drugs are also being studied.

Members of the group during 2014

Lars Bohlin, Professor
Anders Backlund, Professor
Ulf Göransson, Professor

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 course given are Chemistry for biomedicine (15 hp), and 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.

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.

Awards and Appointments 2014

Professor Mats Larhed received the National Swedish Prize in Organic Chemistry (The Ulla and Stig Holmquist Prize), for 2014.

List of Staff

Department of Medicinal Chemistry

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