UPPSALA
UNIVERSITET

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Annual Report 2013

Department of Medicinal Chemistry

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Introduction

The Department of Medicinal Chemistry (DMC) conducts research in three divisions (Analytical Pharmaceutical Chemistry, Pharmacognosy and Organic Pharmaceutical Chemistry) and at the Preclinical PET Platform (PPP). Research activities focus on basic research in drug discovery and development, and the main research programmes are:

- *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.*

Our research achievements are presented later in this annual review.

Governmental support for research and second and third cycle education (FoU) is inadequate, and so we must constantly and actively seek external funding. I'm very pleased to see that our researchers been quite successful in attracting grants during 2013, and it is especially encouraging to see the work of young researchers acknowledged through external funding.

Sunithi Gunasekera received a grant from Swedish Research Link (Research Grant for International Collaboration) for the project 'Discovery of bioactive substances for potential pharmaceutical applications from Sri Lankan medicinal plants'. The aim of the project is to facilitate exchanges of researchers/students between the two countries for scientific collaboration.

Professor Anders Backlund was a co-applicant receiving EU FP7 'Initial Training Network' (ITN) support for the project 'MedPlant'. He is involved in the sub-project 'Phylogenetic exploration of medicinal plants'.

In 2013 the Disciplinary Domain of Medicine and Pharmacy allocated funds to support research within the One Health initiative. Cecilia Alsmark was awarded SEK 700 000 to study a sialidase of bacterial origin in the genomes of trichomonad and trypanosomatide parasites. The long-term aim is to identify potential targets and corresponding inhibitors for new drugs.

Financial support was also given to a new and challenging research area, 'Drugs in the Environment', at the Division of Analytical Pharmaceutical Chemistry. Olof Eriksson and Anna Orlova at PPP also received financial support from the Disciplinary Domain for their research in PET imaging. Eriksson also received grants from *Diabetesfonden* and *Barndiabetesfonden* for his project 'Translational imaging of the human pancreas (TRIUMPH) – new methodologies for understanding the pathologies underlying diabetes'. The aim of the research is to develop a toolbox of methodologies for studying different aspects of the human pancreas in vivo, particularly in terms of the diabetic condition (beta-cell mass, inflammation, etc.).

The government has decided to invest extensively in the research field of Drug Discovery and Development (DDD) through the establishment of SciLifeLab DDD, allocating approximately SEK 40 million/year in the period 2013-2015, thereafter SEK 50 million/year. The foundation of SciLifeLab DDD is a national initiative to conduct early drug discovery in an academic setting, with the aim of identifying and developing early drug candidates. Professor Mats Larhed is the Director of the Medicinal Chemistry Lead ID platform, with 2.5 employees in Uppsala.

The Beijer Laboratory for Drug Discovery, involving the Departments of Pharmaceutical

Biosciences and Medicinal Chemistry was created in September 2013. The Department of Medicinal Chemistry is very grateful for the economic support of 1 million/year for the period 2014-2018 from the Kjell and Märta Beijers Stiftelse.

Professor Anders Karlén devoted a lot of his time in 2013 to coordinating and preparing the ENABLE (European Gram Negative Antibacterial Engine) application for a large EU IMI (Innovative Medicines Initiative) grant. IMI is a public-private partnership between the European Union and the pharmaceutical industry, represented by the European Federation of Pharmaceutical Industries and Associations (EFPIA). The ENABLE project aims to discover and develop new drugs against Gram-negative infections, which are for example responsible for many of the severe and in many cases untreatable infections occurring in hospital environments today. A few weeks ago we were very pleased to hear that IMI had decided to support this research project with a grant of EUR 85 million for the period 2014-2020. We all congratulate Professor Karlén (Leader of the Managing Entity) and his co-workers for this most impressive achievement.

Another funding application under preparation in 2013 was for the major European Institute of Innovation and Technology (EIT), Knowledge and Innovation Community (KIC) call 'Innovation for healthy living and active ageing'. Professor Mats Larhed is coordinating this InnoLIFE consortium application on behalf of Uppsala University. InnoLIFE brings together the needs and potential of citizens with the top talents, creating a world-class innovation ecosystem for healthy living and active ageing. The InnoLIFE grant application will be submitted in September 2014, and we are optimistic it will be approved.

Professor Mats Larhed was a co-applicant receiving EU FP7 'Initial Training Network' (ITN) support for the project 'ARIADME'. He is involved in the sub-project 'A bioisostere approach for the development of antibiotics with optimized ADME properties'.

A few words on the teaching situation, which in recent years has been a concern in terms of the flow of students through the Pharmacy programmes. Teachers are becoming increasingly involved in re-examinations, and in the present economic situation the department is not compensated for the extra work needed to enable students to pass the examinations. A growing number of students accepted on our programmes seem to be inadequately prepared for academic studies in the subjects of mathematics, chemistry and biology. We must try to attract more better-qualified students to accelerate the flow of students through our undergraduate courses, while retaining the high quality of the programmes.

We are improving our teaching skills on the undergraduate programmes, and also streamlining the administration of written examinations by continuously introducing computer-based examinations (CBE) in our courses. In 2013, the Disciplinary Domain of Medicine and Pharmacy decided to prioritise CBE by allocating funds for 150 computers and also to promote CBE software development. Hopefully, we will be introducing CBE on all our courses in 2014. In 2013, we tried to relieve the load on our teachers on undergraduate programmes by using pharmacy students as assistants, and by engaging retired staff on a part-time basis.

The Swedish Higher Education Authority evaluated the quality of the Master of Science in Chemical Engineering programme, and we were very pleased to see it awarded the highest grade, 'Very High Quality'. The evaluation of the Master of Science in Pharmacy programme started in 2013 and will end in 2014. Hopefully, it will also be acknowledged as a programme of very high quality. In addition to preparing for the evaluation, our teachers were involved in revising and developing the new pharmaceutical programmes. It is most important that the work in evaluating and developing new pharmacy programmes is efficient and brings good results, as it diverts valuable time and resources from teaching and research.

Despite the heavy work load, all teaching staff on undergraduate programmes are highly motivated. Anja Sandström was one of ten university lecturers/scholars in Sweden selected for a fellowship in the Swedish Foundation for International Cooperation in Research and Higher Education (STINT) programme, 'Excellence in Teaching'. As a result Associate Professor Sandström spent the autumn term 2013 at Amherst College, one of the leading and selective liberal arts colleges in the US. She participated in the academic life of the college as a faculty member and taught a higher-level course in 'Medicinal Chemistry

and Drug Design'. She also received the teaching award, *Studierådets särskilda pris för studentbemötande* from the Swedish National Association of Pharmaceutical Students in 2013.

DMC has increased its capacity to carry out research and teaching through permanent and temporary recruitments. Torbjörn Arvidsson was appointed adjunct professor in analytical pharmaceutical chemistry and Mikael Hedeland has also joined the Division of Analytical Pharmaceutical Chemistry as Guest Professor for the period 2013-2015, Anna Orlova was appointed senior lecturer at PPP, and the Division of Pharmacognosy recruited Christina Wedén to a 50% junior lectureship position. In 2013, we also recruited Anna Helena Brandhammar as financial administrator and Birgitta Hellsing as course administrator.

On behalf of the Department of Medicinal Chemistry, I welcome our new staff and students, and look forward to working with you in taking our department forwards.

I would also like to take this opportunity to thank those who left our department during 2013 for their excellent contributions over the years. Finally, I would like to thank PhD and post-doctoral students, teachers, researchers and the administrative staff for their excellent work, resulting in the progress made at the Department of Medicinal Chemistry in 2013. It is a most inspiring task to be head of this department.

This Annual Review presents a brief summary of the activities of the Department of Medicinal Chemistry during 2013. 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), and you are more than welcome to contact us personally if you wish to know more.

Uppsala, April 2014
Curt Pettersson, PhD, Professor
Head of Department of Medicinal Chemistry

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Organization

Department of Medicinal Chemistry

Head of Department
Curt Pettersson

Deputy head of Department
Anders Karlén

Department board
Curt Pettersson, chairman
Ann-Marie Benson, technical/administrative130101-130228/Gunilla Eriksson, secretary, technical/administrative
Ulf Göransson, teacher representative
Mats Larhed, teacher representative
Anja Sandström, teacher representative
Albert Elmsjö, graduate student representative
Emilia Gjurovska, undergraduate student representative
Ylva Hedeland, teacher representative, deputy
Anders Backlund, teacher representative, deputy
Sorin Srbu, technical/administrative
Stefan Svahn, graduate student representative

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

Course secretariat
Ann-Marie Benson (130101-130228)
Birgitta Hellsing (130301-tv)
Maj Blad

Computers/IT
Anders Backlund
Jakob Haglöf/Axel Rydevik
Anders Karlén
Sorin Srbu
Sergio Estrada/Olof Eriksson

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
Vt2013
Anders Karlén (50%)
Anja Sandström (50%)
Ht 2013
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
- Evaluation expert for NCR3 UK
- Evaluation expert for Austria Science fund
- Member of the International Editorial Reviewer Board of International Journal of Diagnostic Imaging (IJDI)

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 Uppsala Medical Society

Ulf Göransson
- 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
- Co-chair and member of the organizing committee of the 2nd International Conference of Circular Proteins, Heron Island, Australia
- Member of the Editorial Advisory Board of the journal "Peptidomics"

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

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
- 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 the European Society of Combinatorial Sciences
- 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

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

Christian Sköld
- Member of the Program committee for the Biomedicine program, Faculty of Medicine, Uppsala University
- Chairman of the Equal opportunities group, Department of Medicinal Chemistry, Uppsala University
- System owner of the IT object Research documentation, 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 tandem mass spectrometry (LC-MS/MS) and NMR 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-That means that one enantiomer may be responsible for the therapeutic effect, whereas the other may be inactive or even toxic.

Techniques such as liquid chromatography, supercritical fluid chromatography, capillary electrophoresis as well as mass spectrometry and nuclear magnetic resonance (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 high resolution nuclear magnetic resonance and mass spectrometry
- Analysis of drugs in the environment
- Chiral separation methods
- Capillary electrophoresis for biomedical applications

Members of the group during 2013

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, Associate 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

Publications 2011-2013

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. McEwen I, Elmsjö A, Lehnström A, Hakkarainen B, Johansson M Screening of counterfeit corticosteroid in creams and ointments by NMR spectroscopy J. Pharm. Biomed. Anal. 70; 2012, 245-250

3. 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

4. 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

5. Nilsson LB, The bioanalytical challenge of determining unbound concentration and protein binding for drugs, Bioanalysis 5; 2013, 3033-3050

6. Amini A, Separation of somatropin charge variants by multiple-injection CZE with polybrene/chondroitin sulfate A double-coated capillaries, J. Sep. Sci. 36; 2013, 2686-2690

7. Sänger-van de Griend C, Revival of Capillary Electrophoretic Techniques in the Pharmaceutical Industry, LCGC North America 30; 2012, 954

Agencies that support the work/Funding

Medical Product Agency

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

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

This multidisciplinary project aims to develop, establish and validate analytical methodologies for metabolomics investigations as well as to apply this platform in a diverse set of relevant collaboration projects. We aim to find scientifically reliable analytical systems for detection, identification and quantification of metabolites (=small endogenous molecules) in biological samples derived from cells or biofluids. The literature dealing with metabolomics hasve grown tremendously during the last five years, though minor effort have been put into development of proper analytical platforms capable of handling a diverse set of matrices. As the field is still considered to be in its youth, there is a critical need for well-constructed sampling protocols and analytical platforms for future progression.

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. Recent publications have concluded that a combined use of NMR spectroscopy and MS provide beneficial synergies for metabolomics purposes. This approach will be usedis implemented throughout the projects as the department and included collaborators have extensive theoretical and practical knowledge of both systems as well as instrument access to them.

The analytical methodology is being developed and evaluated in several interesting ongoing studies through established collaborators found at Uppsala University (UU) and Karolinska Institute (KI). Of particular focus are metabolomic investigations in cancer research where the division is currently engaged in seven different projects. In collaboration with the Department of Medical Sciences (UU), the division is focusing on 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). In collaboration ofwith 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 2011-2013

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

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. Few of these methods focus on the metabolites, which can be just as equally or even more potent than the parent compound. 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.

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

Our aim is to develop validated analytical methods where the risk for sample contamination is reduced. With these methods we intend to detect identify and quantify pharmaceuticals and metabolites as well as their stereoisomers that are of particular interest from an environmental point of view. Our second aim is to analyze and identify metabolic pathways for pharmaceuticals in the environment. For this goal, 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 pharmaceuticals.

The ongoing project is focusing on the degradation of pharmaceuticals in β-blocking agents in Californian wetlands, using β-blocking agents as model substances. By comparing different wetland types and conditions, the degradation pathway and rate is being estimated. This work includes metabolite searching, quantification using labeled standards, and developing and application of chiral separation methods to estimate the enantiomeric fraction. Generally, solid phase extraction is used for targeted sample analysis due to the complex matrix, and both high and low resolving MS for detection. The separation system is generally LC, but for chiral applications SFC is also used.

Publications 2011-2013

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

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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

Chiral separation methods

Curt Pettersson, Ylva Hedeland, Monica Johansson, Niklas Tyrefors, Olle Gyllenhaal, Victoria Barclay, Alexander Hellqvist

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 2011-2013

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

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Capillary electrophoresis for biomedical applications

Curt Pettersson, Ylva Hedeland and Alexander Hellqvist (PhD student)

The aim with this project is to develop methods for biomedical applications in veterinary medicine based on capillary electrophoresis and it is performed in cooperation with Dr Reidun Heiene at the Norwegian School of Veterinary Science (Oslo, Norway) and Blue Star Animal Hospital (Gothenburg, Sweden) and Siegrid De Baere at the Dept. of Pharmacology, Toxicology and Biochemistry, Ghent University.

The emphasis has been to develop methods for determination of renal function and to enable differentiation between acute and chronic renal failure. A simple and reliable method for determination of iohexol, a glomerular filtration rate (GFR) marker, in plasma has been developed and validated. An additional objective is to develop a method for analysis of haemoglobine subtypes in order to enable differentiation between acute and chronic renal failure.

Publications 2011-2013

1. 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 2013

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

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 åmetabolites may be more active than the parent compound, or even toxic, it is of outmost 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 2013

Ulf Bondesson, Adjunct Professor
Mikael Hedeland, Associate Professor
Axel Rydevik, PhD student
Nina Klasson, student
Johanna Wedin, student
Anna Hellqvist, student

Publications 2011-2013

1. E. Bergman, E.M. Matsson, M. Hedeland, U. Bondesson, L. Knutson, H. Lennernäs: Effect of a single gemfibrozil dose on the pharmacokinetics of rosuvastatin in bile and plasma in healthy volunteers, *Journal of Clinical Pharmacology*, 50; 2010, 1039-1049.

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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 proteases 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) as well as the infectious diseases malaria and tuberculosis. 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, Marc Stevens, Luke Odell, Ulrika Rosenström, Fredrik Svensson, Christian Sköld, Puspesh Upadhyay

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, Jean-Baptiste Veron, Luke Odell, Mathias Alterman, Jonas Rydfjord, Jonas Sävmarker

Introduction: The role of the AT2 receptor is not jet 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 2013

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Charlotta Wallinder, Researcher
Mathias Alterman, Guest Researcher
Ulrika Rosenström, Researcher
Karin Engen, PhD student
Fredrik Svensson, PhD student
Puspesh Upadhyay, PostDoc

Publications 2011-2013

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Reviews 2011 – 2013

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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, Alejandro Trejos, Jean-Baptiste Veron, 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 s 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, Alejandro Trejos, Johan Gising, Patrik Nordeman, Ashkan Fardost, Linda Åkerbladh, Hitesh Motwani, Marc Stevens

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, Alejandro Trejos, Luke Odell, Jonas Sävmarker, Patrik Nordeman, Peter Nilsson, Ashkan Fardost, Jonas Rydfjord, Rajendra Mane, Jean-Baptiste Veron, 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, Alejandro Trejos, Patrik Nordeman, 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.

Members of the group during 2013

Mats Larhed, Professor
Anders Hallberg, Professor
Anders Karlén, Professor
Linda Axelsson, PhD student
Johan Gising, Research Associate
Luke Odell, Assoc. Professor
Christian Sköld, Research Associate
Jonas Sävmarker, Research Associate
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Marc Stevens, PhD student
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Heterocyclic Chemistry

Luke Odell, Marc Stevens, Rajiv Sawant

Background: Heterocycles are of extreme importance in drug discovery and medicinal chemistry. The vast majority of marketed drugs are either heterocycles or contain heterocyclic ring systems. Thus, new methodologies for the construction or functionalization of hetereocyclic scaffolds are highly sought after. 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.

Publications 2011-2013

1. C. Sköld, J. Kleimark, A. Trejos, L. R. Odell, S. O. Nilsson Lill, P-.O. Norrby, M. Larhed: Transmetallation Versus β-Hydride Elimination: The Role of 1,4-Benzoquinone in Chelation-Controlled Arylation Reactions with Arylboronic Acids.
   Chem. Eur. J. 18, 2012, 4714-4722.

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   One-pot, two-step, microwave-assisted palladium-catalyzed conversion of aryl alcohols to aryl fluorides via aryl nonaflates.
   J. Org. Chem. 78, 2013, 4184–4189

3. F. Svensson, R. S. Mane, J. Sävmarker, M. Larhed, C. Sköld
   Theoretical and experimental investigation of palladium(II)-catalyzed decarboxylative addition of arenecarboxylic acid to nitrile.
   Organometallics 32, 2013, 490–497

4. J. Rydfjord, F. Svensson, A. Trejos, P. J. R. Sjöberg, C. Sköld, J. Sävmarker, L. R. Odell, M. Larhed
   Decarboxylative palladium(II)-catalyzed synthesis of aryl amines from aryl carboxylic acids: development and mechanistic investigation
   Chem. Eur. J. 19, 2013, 13803–13810

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 2013

Anders Karlén, Professor
Mats Larhed, Professor
Hiba Alogheli, PhD Student
Linda Åkerbladh, PhD Student
Martin Lindh, PhD Student
Bobo Skillinghaug, PhD Student
Fredrik Svensson, PhD Student
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Hitesh Motwani, Postdoctoral Fellow
Luke Odell, Research Associate
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Publications 2011-2013

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Synthesis, Biological Evaluation and X-Ray Crystallographic studies of Imidazo[1,2-a]pyridine-based Mycobacterium Tuberculosis Glutamine Synthetase Inhibitors. Med. Chem. Commun. 3 (2012), 620-626.

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Vinnova 1 600 000 SEK (2011)

Design and synthesis of Mtb Glutamine Synthetase inhibitors

Anders Karlén, Mats Larhed, Johan Gising, Martin Lindh, and 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 and 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, Martin Lindh, Bobo Skillinghaug, Fredrik Svensson, Shyamraj Dharavath, Christian Sköld and 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.

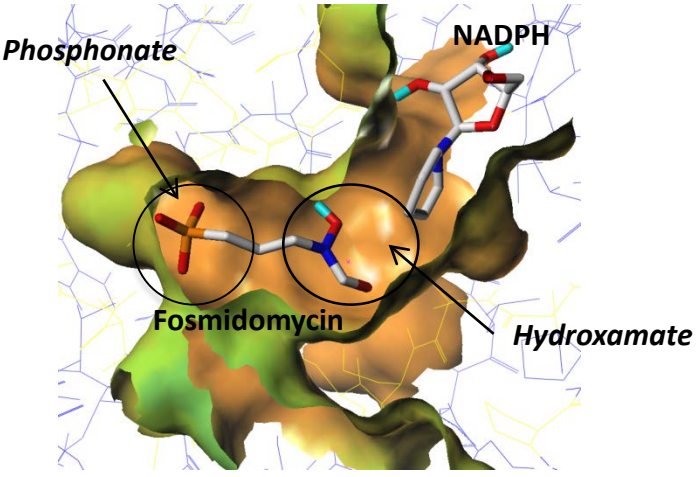

Figure 1. Crystal structure of fosmidomycin bound to *Mtb* DXR. Only the active site is shown for clarity and only part of the NADPH molecule is shown.

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, Jonas Lindh, Johan Gising, Hiba Alogheli, Gunnar Lindeberg

We have recently initiated a project to investigate the possibility that proteases may be useful antituberculosis drug targets (Vinnova Sambio grant together with Medivir). 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 areas of 3D-QSAR and 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 2013

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

Publications 2011-2013

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, 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: Design and Synthesis of Hepatitis C Virus Protease Inhibitors and Neuropeptide Mimetics

Research Group Leader: Anja Sandström

Peptides are major players in physiological processes both in mammalians and in microorganisms such as viruses. The so called neuropeptides are for example a large and important group of neurotransmitters that often acts in a modulatory way in the nervous system. Peptides can serve as valuable research tools in the first phases of drug discovery projects and for the study of biological mechanisms behind various diseases. However, peptides are not suitable as pharmaceuticals intended for oral administration due to the inherited drawbacks related to the peptide structure as rapid degradation by proteolytic enzymes and low bioavailability. 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 molecules/ peptidomimetics *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 novel peptidomimetics and peptidomimetic scaffolds; with special focus on protease inhibitors of hepatitis C virus and mimetics of the neuropeptide Substance P 1-7.

Members of the group during 2013

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
Sanjay Borhade, Postdoctoral Fellow
Ankur Pandey, Postdoctoral Fellow
Anna Skogh, PhD student

Publications 2011-2013

1. M. Ohsawa, A. Carlsson, A. Megumi, T. Koizumi, Y. Nakanishi**,** R. Fransson, A. Sandström, M. Hallberg, F. Nyberg and J. Kamei: The effect of Substance P1-7 amide on nociceptive threshold in diabetic mice.
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   Bioorg. Med. Chem. 2011, 19, 4917-4927.

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   Organic Letters (2013), 15, 1056-1059.

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   J. Med. Chem (2013). DOI: 10.1021/jm301887f

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   J. Med. Chem., 2013, *56* (12), pp 4953–4965

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10. A. Skogh, R. Fransson, C. Sköld, M. Larhed, A. Sandström*. Aminocarbonylation of 4-iodo-1H-imidazoles with an amino acid amide nucleophile: Synthesis of constrained H-Phe-Phe2 analogues.
    J. Org. Chem. 2013, 78, 12251-12256

The Hepatitis C Project

Anja Sandström, Eva Åkerblom, Anders Karlén, Anna Karin Belfrage, Johan Gising, Hiba Alogheli, Sanjay Borhade.

The interest in antiviral therapies has increased dramatically the last decades as shown by several successful market approvals in recent times. One important reason for this is that vaccines remain unavailable for many severe infectious diseases, including malaria, human immunodeficiency 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, as in the highly active antiretroviral therapy (ART) that was introduced in the mid-90s and which had a tremendous effect on AIDS mortality. 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 was recently augmented with two peptidomimetic HCV NS3 protease inhibitors (telaprevir and boceprevir). However, even though extensive efforts have been made to develop more powerful next-generation HCV NS3 protease inhibitors, the long term success of this drug class are challenged by the rapid 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.

We have developed several potent protease inhibitors of HCV NS3 over the years. The major achievements of our previous work were firstly the identification of C-terminal acylsulfonamides as bioisosteric replacements of the commonly used C-terminal carboxylic acid in product-based inhibitors, and secondly the discovery of 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 into 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 the wild type protease 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 developing novel carboxylic acid bioisosteres, 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 spinal cord have been identified. Even though the intriguing effects of SP_{1-7} have been known for quite some time SP_{1-7} has not previously been addressed in a medicinal chemistry program. Our early aims of this project was to develop stable and bioavailable peptidomimetics of SP_{1-7} be used as research tools in functional animal studies for a more thorough understanding of the physiological function of $SP_{1-7,}$ including identification of its macromolecular target.Our initial efforts in this area included a thorough SAR study of the binding of SP_{1-7} and endomorphin-2 (EM-2) to the SP_{1-7}-binding site by means of Ala-scans, truncation studies and C- and N-terminal modifications of the two target peptides. From this we concluded that only the C-terminal part was crucial for the affinity. Moreover, C-terminal amidation potentiated the ligands in both *in vitro* and *in vivo* models. Altogether, the SAR studies led to the remarkable discovery of a small dipeptide ligand having equal affinity as endogenous heptapeptide ligand SP_{1-7}. SP_{1-7}, SP_{1-7}-amide and the dipeptide further demonstrated potent analgesic effect on pain of neuropathic origin in *in vivo* models. Chronic neuropathic pain is an underrecognized and undertreated diagnosis which constitutes a major public health problem and a vast economic burden to society. There is a great need for new therapies specific for neuropathic pain. Several new types of less

basic and constrained amino acid/dipeptide mimetics, including multidecorated heteroaryls, as well as synthetic protocols, are currently under preparation in order to improve the pharmacokinetics properties of the ligands.

Publications from Division members in 2011-2013, unrelated to the projects above

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ChemMedChem, 7 (2012) 396-399.

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J. Med. Chem. 55 (2012), 6866-6880. DOI: 10.1021/jm300623u

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Microwave-Assisted synthesis of Guandidin Organocatalysts Bearing a Tetrahydroisopuinline Framwork and Their Evaluation in Michael Addition Reactions.
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Pentacycloundecane-diol-Based HIV-1 Protease Inhibitors: Biological Screening, 2D NMR, and Molecular Simulation Studies. ChemMedChem. 7 (2012) 1009-1019. DOI: 10.1002/cmdc.201100512

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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.

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 2013.

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 β-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 2013

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

Ola Åberg, Scientist
Ewa Hellström-Lindahl, Associate Professor
Mats Larhed, Professor
Jennie Malmberg, PhD student
Patrik Nordeman, PhD student
Anna Orlova, Associate Professor
Ulrika Rosenström, Guest Lecturer
Ramkumar Selvaraju, PhD student
Marc Stevens, PhD student
Alf Thibblin, Assoc. Prof.
Zohreh Varasteh, PhD student
Irina Velikyan, Associate Professor

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, a clinical PET/CT scanner and a Hamamatsu large animal scanner. Collaboration with the PET center at Uppsala University Hospital ensures rapid translation from preclinical to clinical studies.

Members of the group during 2013

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 2011-2013

1. O. Eriksson, A. Sadeghi, B. Carlsson, T. Eich, T. Lundgren, B. Nilsson, T. Totterman, O. Korsgren, A. Sundin. Distribution of adoptively transferred porcine T-lymphoblasts tracked by ^{18}F-2-fluoro-2-deoxy-D-glucose and position emission tomography. Nucl Med Biol 38; 2011, 827-833.

2. M. Jahan, O. Eriksson, P. Johnström, O. Korsgren, A. Sundin, L. Johansson, C. Halldin. Decreased defluorination by using the novel beta cell imaging agent [^{18}F]FE-DTBZ-d4 in pigs examined by PET. EJNMMI Res 1; 2011, 33.

3. O. Eriksson, F. Carlsson, E. Blom, A. Sundin, B. Långström, O. Korsgren, I. Velikyan. Preclinical evaluation of a ^{68}Ga-labeled biotin analogue for applications in islet transplantation. Nucl Med Biol 3*;* 2012, 415-21.

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Reviews 2011-2013

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2. D. Espes, O. Eriksson, J. Lau, P.O. Carlsson. Striated muscle as implantation site for transplanted pancreatic islets. J Transplant 2011; 2011, 352043.

Agencies that support the work/Funding

1. Diabetesfonden
2. Barndiabetesfonden,
3. ExoDiab
4. Tore Nilssons Stiftelse
5. JDRF
6. EFSD

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 jet 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 2013

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

Development of 5-Fluoro-[β-11C]-L-tryptophan as a functional analogue of 5-hydroxy-[β-11C]-L-tryptophan for PET studies of neuroendocrine tumours and □-cell mass in pancreas

Research Group Leader: Gunnar Antoni and Olof Eriksson

5-Hydroxy-[β-^{11}C]-L-tryptophan ([^{11}C]HTP) is an established positron emission tomography (PET) imaging agent for neuroendocrine tumors (NETs). It has also been used for other clinical research purposes in neurology and diabetes. However, its widespread use is limited by the short physical half-life of the radionuclide and a difficult radiosynthesis. Therefore, a fluorine-18 labeled analogue, 5-[^{18}F]fluoro-L-tryptophan, ([^{18}F]FTRP) has been proposed as a functional analogue. There is no published method for the synthesis of L-[^{18}F]FTRP. We have therefore developed a synthesis of 5-fluoro-[β-^{11}C]-L-tryptophan ([^{11}C]FTRP), based on the existing chemo-enzymatic method for [^{11}C]HTP and evaluated the potential usefulness of radiolabeled FTRP in direct comparison with [^{11}C]HTP.

Members of the group during 2013

Gunnar Antoni
Olof Eriksson
Ramkumar Selvaraju
Veronika Asplund
Sergio Estrada

Publications

1. Olof Eriksson, Ramkumar Selvaraju, Beatrice Borg, Veronika Asplund, Sergio Estrada, Gunnar Antoni. 5-Fluoro-[β-^{11}C]-L-tryptophan is a functional analogue of 5-hydroxy-[β-^{11}C]-L-tryptophan in vitro but not in vivo. Accepted for publication in *Nuc Med Biol Jan 2013*

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 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 2013

Gunnar Antoni, Associate Professor
Veronika Asplund, Research Engineer

Synthesis and preclinical evaluation of a 11C-labelled libiguin

Research Group Leader: Gunnar Antoni

Libiguins are a new class of compounds with potential central effect on the regulation of sexual behaviour. Clinical trials have shown positive effects of libiguins on sexual dysfunctions such as impotence, restoring the sexual function, which also has been confirmed in animal studies where the frequency of mating significantly increases after libiguin administration. We are currently investigating if ^{11}C-labelled libiguins could be used to identify the areas in the brain where a central effect could be mediated, for example, through a so far unidentified receptor or enzyme system.

Members of the group during 2013

Gunnar Antoni, Associate Professor
Sergio Estrada, Scientist
Alf Thibblin, Associate Professor

Autoradiography study of angiogenesis in abdominal aortic aneurysm with [18F]fluciclatide – an □V□3 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 2013

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

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 (A □). 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 2013

Sergio Estrada, Scientist
Gunnar Antoni, Associate Professor
Ewa Hellström-Lindahl, 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, Marie Svedberg, Mats Larhed, Alf Thibblin, Håkan Hall

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 the of complex chemical signalling systems that is 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.

Synthesis and radiolabelling of PET tracers for the study of Alzheimer's disease and trauma targeting the □-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- labelleld tiophene derivatives as tracers for the study of Alzheimer’s disease and systemic amyloidosis.

Gunnar Antoni, Patrik Nordeman, Peter Nilsson, Per Hammarström, 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 A □deposits as well as prefibrillar A □ assemblies a 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 □ chains. The i
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.

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 and 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 and 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,

Project description

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.

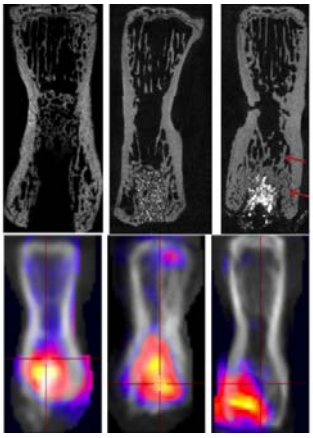

Figure 1: a rat-tail vertebral defect filled with bone substitutes. The lower picture show the osteoblast activity in the defect studied in SPECT with a radioactive tracer.

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 □□□ 3 receptors.

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

Research Group Leaders: Mats Larhed, 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 2013

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

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, 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 A deposits as well as prefibrillar A assemblies and 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 2013

Gunnar Antoni, Associate Professor
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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 2013

Anna Orlova, Associate Professor
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Maria Rosestedt, MS student, PhD student

Thesis defended

Jennie Malmberg, 2013-11-08, Preclinical development of imaging agents for HER2 expression in prostate cancer using radiolabeled affibody molecules.

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Agencies that support the work/Funding

Swedish Cancer Society (Cancerfonden), 500 000 SEK
Swedish Research Council (Vetenskapsrådet), 900 000 SEK

Development of in vitro predictive assay for renal and hepatic uptake of conjugates for radionuclide molecular targeting.

Radionuclide-based diagnostic and therapy are rapidly developing areas of medicine, particularly in oncology. A promising direction in nuclear medicine is the development of radionuclide molecular targeting (RMT) agents detecting the presence of molecular biomarkers on primary or metastatic lesions. Information of molecular biomarkers can be utilized later for selection of patients for biomarker-specific therapy, e.g. immunotherapy. The use of RMT agents, which are labelled with cytotoxic nuclides (e.g. beta- or alpha-emitters) would permit direct radionuclide therapy of tumours. Potent imaging and/or therapeutic agent in nuclear medicine must have high and stable specific uptake in lesion (primary tumours or metastasis) and quick clearance from healthy organs. Imaging RMT agents with such features would provide better imaging contrast and, consequently, better imaging sensitivity. Therapeutic RMT agents would decrease radiation burden to patients and provide broader therapeutic window.

Biodistribution properties of an RMT agent depends on many factors, e.g. nature of targeting protein, its specificity to the target, its charge and lipophilicity and a labelling method, and cannot be predicted a priori. Therefore developing of RMT includes biodistribution studies in laboratory animals. Prediction of a high liver and kidney uptake of an RMT agent would enable exclude this agent from consideration at early stage. The goal of this project is the development of *in vitro* assays for prediction of liver and kidney uptake of potential RMT agents. Achieving of this goal would replace the animal studies by *in vitro* assay and reduce a number of animals, which are sacrificed for development of RMT tracers.

For the moment, there are a large number of *in vitro* assays, which can predict tumour-targeting properties of RMT conjugates (affinity, specificity, cellular processing and retention). Such assays have been widely used in our research concerning development of targeting conjugates. At the same time, *in vitro* assays for prediction of hepatic and renal assays are missing. Analysis of the literature indicates that development of such assays is feasible. For example, opossum kidney (OK) cell line derived for proximal tubule has been used for elucidation of renal uptake mechanism for ^{111}In-labelled octreotide. A number of studies on physiology, toxicology and pharmacology utilised immortal hepatoma cell lines,as *in vitro* models. These studies show that molecular mediators of uptake (scavenger receptors, transporters, channels) remained to be expressed in renal proximal tubule- and hepatocyte-originating cell lines *in vitro*. This creates pre-conditions for development of *in vitro* assays for uptake and retention of radiolabelled RMT conjugates in liver and kidneys.

Members of the group during 2013

Anna Orlova, Associate Professor
Jennie Malmberg, PhD student
Zohreh Varasteh, PhD student

Publications 2011-2013

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Agencies that support the work/Funding

Swedish Research Council (Vetenskapsrådet), 900 000 SEK

Development of radiolabelled affibody molecules for radionuclide molecular imaging of HER3 expression in malignant tumours. A diagnostic tool for patient stratification in HER3 targeted therapy.

HER3 (human epidermal growth factor type 3) is a transmembrane tyrosine kinase receptor that is overexpressed in several types of tumours (e.g. breast, prostate, lung and ovarian carcinomas). Overexpression of HER3 is associated with e.g. resistance of breast cancer to tyrosine kinase inhibitors and to the antibody trastuzumab, progression to androgen independence in prostate cancer, and resistance to chemotherapy in gastric cancer and head and neck squamous cell cancer. Several HER3-targeting drugs (antibodies as well as signalling inhibitors) are currently in clinical trials. Correct patient stratification is essential for successful clinical development of these drugs and for personalizing cancer therapy in routine clinical practice. We propose to use affibody molecules, a novel kind of targeting proteins, for in vivo molecular imaging of HER3 expression. Affibody molecules are small (6.5-7 kDa) affinity proteins with demonstrated capacity for molecular imaging of several cancer-associated molecular targets in pre-clinical studies (HER1, HER2, IGF-1R). Two microdosing clinical studies (one at UAS) have confirmed favourable imaging properties of anti-HER2 affibody molecules in patients.

The goal of the project is to establish a methodology for radionuclide molecular imaging of HER3 expression in vivo, resulting in more personalized HER3-targeting therapy. To reach this goal, following sub-goals should be achieved:

- Establishing of in vitro and in vivo models for study of HER3 targeting. Successful characterisation of the targeting conjugate requires appropriate in vitro and in vivo models with high and stable expression of target. On this stage we are planning to characterise cell lines for HER3 expression (Bmax) and establish reliable murine xenograft models with a high reproducible tumour take. Xenografts with both high and low levels of HER3 expression will be established to investigate capacity of imaging agents for discrimination of expression levels in vivo.;

- Labeling optimization, in vitro and in vivo characterisation of new anti-HER3 conjugates, including validation of their specific targeting of HER3. Several labelling methods will be applied with the aim to find stable in vivo radioisotope attachment to the anti-HER3 affibody molecule. Both PET and SPECT radioisotopes will be used for development of radiotracers to make them suitable for both imaging techniques. Labels with different biological behaviour will be tested. After labelled anti-HER3 affibody molecules have been developed and demonstrated appropriate in vitro targeting properties, they will be studied for biodistribution

pattern and in vivo tumour targeting. We plan to study: influence of labelling on the pharmacokinetics of the targeting conjugate, including questions of blood clearance, accumulation in normal tissues, specificity of tumour accumulation, level of tumour uptake and excretion of radiocatabolites; influence of injected protein dose and timing on the sensitivity of HER3 imaging; possibility to discriminate between xenografts with low and high HER3 expression in vivo by optimization of injected protein dose; dosimetry up-scaling from mice to men.

Successful accomplishment of the project would create a HER3-imaging tool that will be crucial for patient stratification in trials using HER3-inhibitors. Ultimately, a broad implementation of HER3-imaging affibody molecules would provide critical information determining an optimal treatment of e.g. breast cancer in clinical routine. The breast cancer treatment will be more personalized in this way. One might expect the use of HER3 imaging in other types of cancer e.g. in prostate cancer for detection of onset of androgen independence. The results of the project should provide additional evidences for broader application of radionuclide imaging for making cancer treatment more personalized. Knowledge obtained during this project can be used for development of radionuclide imaging probes for other molecular targets in cancer.

Members of the group during 2013

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

Agencies that support the work/Funding

Faculty of Pharmacy, Uppsala University, 750 000 SEK

Publications 2011-2013

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Radiolabelling technology

Development of methods for labelling with synthesis with 11CO

Research Group Leader: 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.

Members of the group during 2013

Gunnar Antoni, Associate Professor
Mats Larhed, Professor
Patrik Nordeman, PhD student

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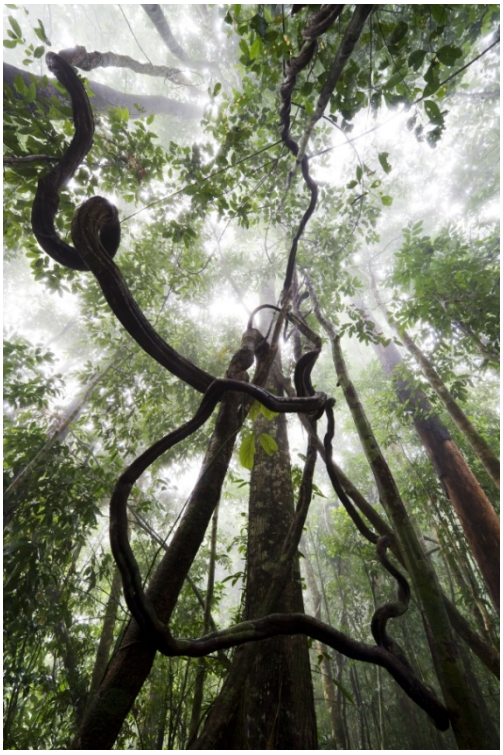
2. I. Velikyan. Positron emitting [68Ga]Ga-based imaging agents: chemistry and diversity. Med Chem 7; 2011, 345-379.

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 proven science, 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

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* 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.

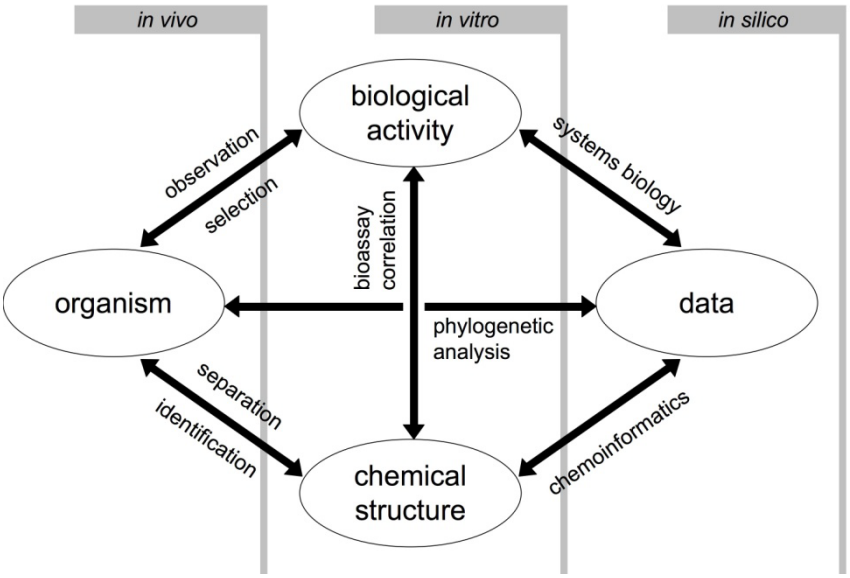

Figure 2. The interdisciplinary nature of pharmacognosy is demonstrated by the explanatory model above (Figure by S. Larsson)

Chemistry and Biology of Ultra Stable Proteins

Research Group Leader: Ulf Göransson

Our research interest lies at the interface between chemistry and biology, and reflects our fascination of natural products and possibilities these molecules represent. In particular, our research is focused on peptides of natural origin, their discovery, biological effects, biochemistry, structure, and, lately, towards peptide chemical design and synthesis. The overall aim of our research is to develop naturally occurring peptides into compounds useful for applications in medicine or biotechnology, and to develop general methods to do so.

The backbone-cyclized plant proteins named cyclotides are central to our research. These compounds 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 3). 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. Their exceptional chemical and biological stability also favors their applications in drug discovery, where they may be used as carriers of less stable peptide sequences.

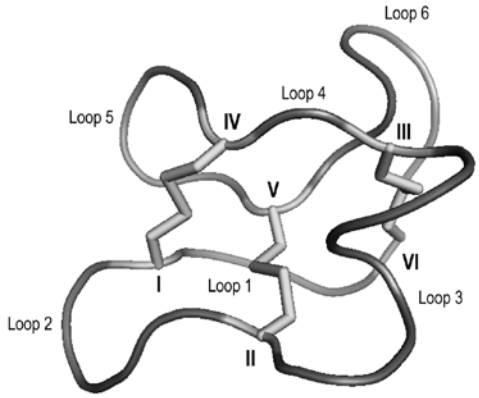

Figure 3. The cyclotide backbone. Note the circular backbone and the cystine knot that define the cyclic cystine knot (CCK) motif. The variable loop regions (marked 1-6) between the cysteines (marked I-VI) are targets for protein engineering in this project. The CCK motif is able to harness a number 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.

Lately, the group has made significant contributions to the field of cyclotide synthesis and folding, and we are building expertise in biophysical studies of membrane interactions. Building on our previous knowledge and the methodology that we have developed, we are now moving into the direction of design and applications of cyclotides as enzyme inhibitors and antimicrobial agents. However, as our research group is expanding so are the research interests: today they include the chemical biology of other peptides, for example structural design of antimicrobial peptides using, and we have recently started to exploit the possibilities given by next generation sequencing for peptide discovery. Some of the research highlights during the year are summarized below in Figure 4.

Our collaboration with professor Hesham El-Seedi, El Menoufia University, Egypt, and Professor Anna-Karin Borg-Karlsson, Royal Institute for Technology, Sweden, has continued. At Uppsala, the collaboration with Professor Björn Hellman has continued, and now focuses on the antimutagenic effects of a Mongolian medicinal plant (the project of Delgerbat Boldbataar). Sohaib Malik has started as a shared PhD student with half his time at Department of Medical Biochemistry and Microbiology in the lab of Prof Dan I Andersson. We collaborate with Håkan Andersson at Linnéuniversitetet and Dr Malin Strand at Göteborgs University about peptide toxins; and we have started collaboration with Dr Per-Johan Jakobsson at the Karolinska Institute.

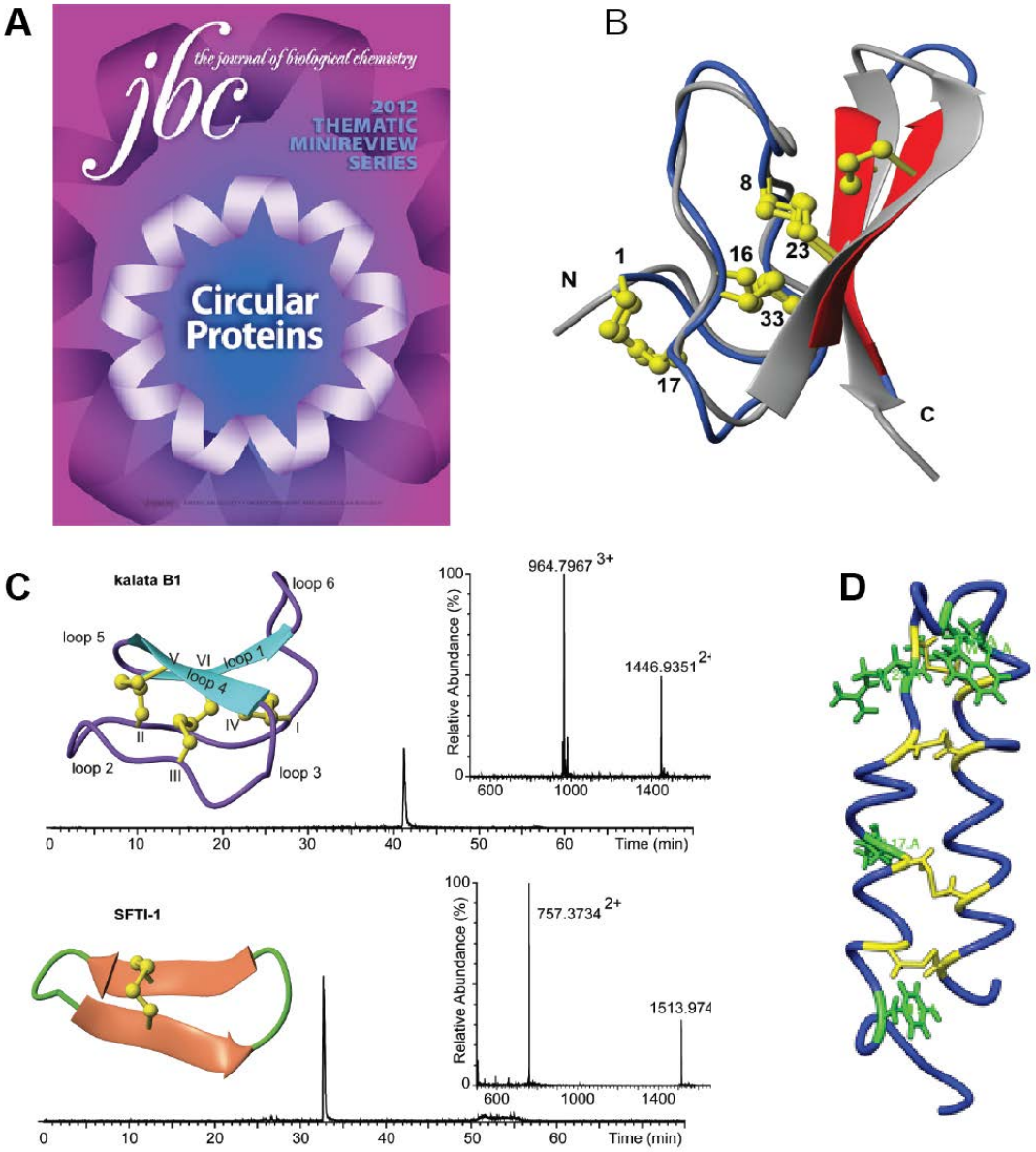

Figure 4. Some research highlights 2012. A) The group contributed with one article in the thematic minireview series on circular proteins published by J Biol Chem (Göransson et al, JBC 2012) B) The antimicrobial peptide isolated from the cactus *Echinopsis pachanoi* has almost an identical structural as some spider toxins. C) We have successfully adapted the Dawson method for native chemical ligation to cyclisation of cyclotides and circular trypsin inhibitors (Gunasekera et al). D) This 55-residue peptide toxin was successfully synthesized and folded.

Internationally, UG is now assistant supervisor of Błażej Ślązak at the Jagiellonian University, Krakow, Poland. Main supervisor is Prof Elżbieta Kuta, and his subject is cyclotides in plants and plant cell cultures of endangered Viola species. 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. Lastly, we have had a continued good collaboration with Drs Johan Rosengren and Richard Clark, and Prof David Craik at the University of Queensland, Australia.

During 2012, six Master students from the Pharmacy Programme, the Uppsala Graduate School for Biomedical Research, and the Master Programme for Infectious Biology have been involved in our research, and one student from the Summer Research School (SOFOSKO).

We have participated with oral and poster presentations at the International Conference of Natural Products Research (New York), the 32nd European Peptide Symposium (Athens) and the 2nd International Conference of Circular Proteins (Heron Island, Australia). UG co-chaired the Circular Protein conference.

A project grant was secured from VR Science and Technology (NT) of 1800 kSEK for the period 2013-2015 for research on circular proteins (UG); SG was supported during 2012 by a postdoc grant from Carl Tryggers Foundation; and AS secured a postdoc grant from Svenska Läkaresällskapet of 108 kSEK. In addition, research on peptides is also a big part of the Division's part in the FP7 Bluegenics consortium. With the support of the Ahlquist foundation, UG spent 3 months on a short sabbatical in the lab of Drs. Johan Rosengren and Richard Clark at the University of Queensland.

Lastly, Mariamawit Yonathan Yeshak succesfully defended her thesis in April. Currently, she is working as a teacher and researcher at the School of Pharmacy, Addis Ababa University.

Members of the group during 2013

Ulf Göransson, PhD, Associate Professor
Sunithi Gunasekera, PhD
Adam Strömstedt, PhD
SungKyu Park, MSc, PhD student
Sohaib Malik, MSc, PhD student
Mariamawit Yonathan Yeshak, MSc, PhD student (defended her thesis in April)
Delgerbat Boldbaatar, MSc, PhD Student guest from National University of Mongolia
Błażej Ślązak, MSc, PhD student
Erik Jakobsson, MSc, Research Assistant (June-Dec)
Camilla Eriksson, MSc, Research Assistant (June-Aug)
Taj Muhammad Khan, MSc, Research Assistant (June-Dec)
Debashish Roy, MSc, Research Assistant (Oct-Dec)

Publications 2011-2013

1. Burman R, Gruber CW, Rizzardi K, Herrmann A, Craik DJ, Gupta MP, Göransson U (2010) Cyclotide proteins and precursors from the genus Gloeospermum: filling a blank spot in the cyclotide map of Violaceae. Phytochemistry, 71(1): 13-20.

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11. Burman R,* Herrmann A,* Tran R, Kivelä J-E, Lomize A, Gullbo J, Göransson U (2011) Cytotoxic potency of small macrocyclic knot proteins: Structure-activity and mechanistic studies of native and chemically modified cyclotides. Organic & Biomolecular Chemistry. 9: 4306-4314. *Contributed equally.

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1. El-Seedi HR, El-Barbary MA, El-Ghorab DMH, Bohlin L, Borg-Karlsson AK, Göransson U, Verpoorte R. (2010) Recent insights into the biosynthesis and biological activities of natural xanthones. Current Medicinal Chemistry, 17: 854-901.

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4. Bohlin L, Alsmark C, Göransson U, Klum M, Wedén C, Backlund A (2011) Strategies and methods for a sustainable search for bioactive compounds. In Bioactive Compounds from Natural Sources, Second Edition: Natural Products as Lead Compounds in Drug Discovery. Edited by Tringali C. CRC Press, Taylor & Francis Group, LLC, Boca Raton, FL. ISBN 978-1-4398-2229-6

5. 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.

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Dissertations 2013

1. Yeshak, Mariamawit Yonathan, “Cyclotides: Tuning Parameters Toward Their Use in Drug Design”. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISBN 978-91-554-8307-4

Agencies that support the work/Funding

Swedish Foundation For Strategic Research, Programme for Future Research Leaders, 1700 000 SEK
Swedish Research Council, NT, 850 000 SEK
Carl Tryggers Foundation, 240 000 SEK

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 2013

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

Publications 2011-2013

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

Reviews 2011-2013

1. L. Bohlin, U. Göransson, C. Alsmark, C. Wedén, A. Backlund: Natural products in modern life science. Phytochemistry Reviews. 9(2*);* 2010, 279-301.

2. Bohlin L, Alsmark C, Göransson U, Klum M, Wedén C, Backlund A (2011) Strategies and methods for a sustainable search for bioactive compounds. In Bioactive Compounds from Natural Sources, Second Edition: Natural Products as Lead Compounds in Drug Discovery. Edited by Tringali C. CRC Press, Taylor & Francis Group, LLC, Boca Raton, FL. ISBN 978-1-4398-2229-6

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 world-wide, a web-site 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.

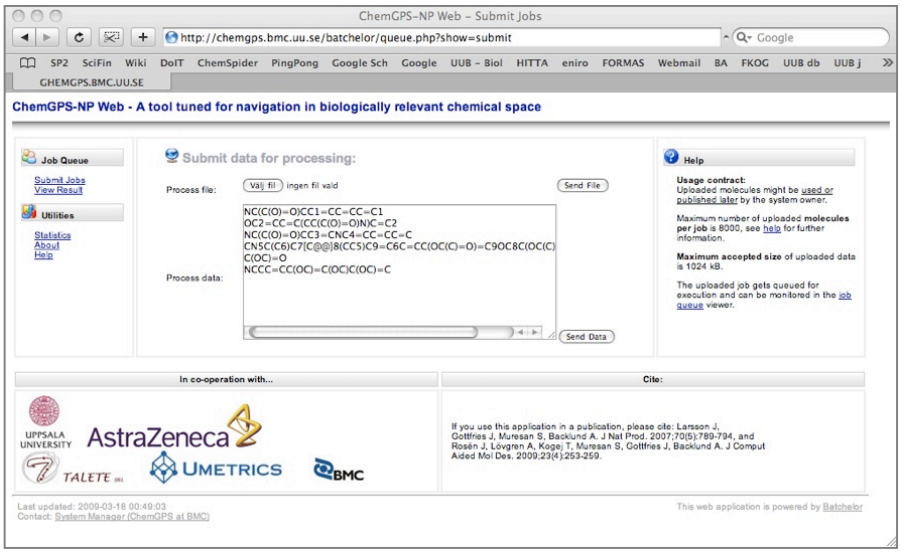

Figure 5. Web interface for ChemGPS-NPweb.

Since the launch in May 2008 more than 7 million compounds originating from more than 4200 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.

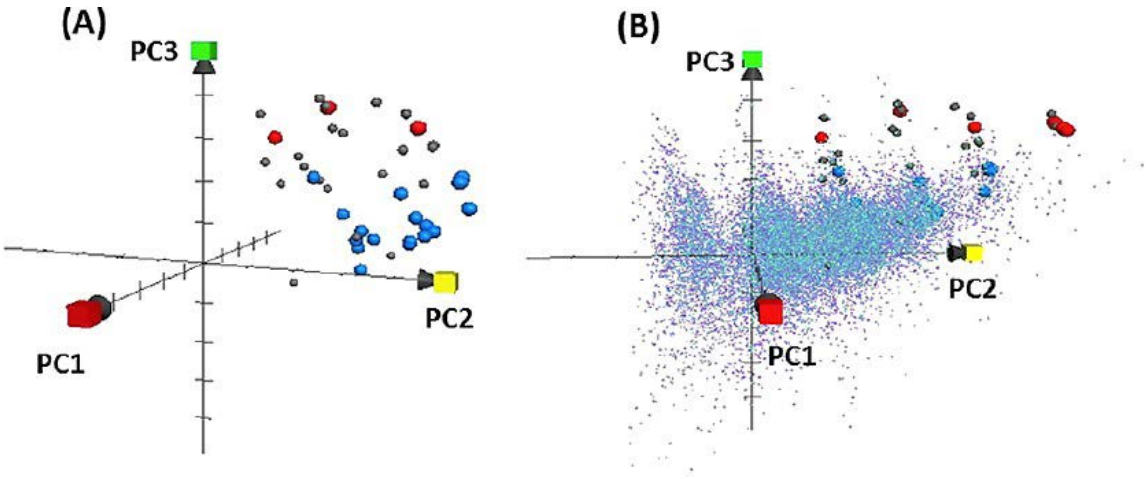

Figure 6. In 6A we can see a set of cytotoxic compounds plotted in chemical property space. Those coloured in red indicates highly potent compounds, those in blue less potent, and the gray compounds with low or intermediate effect. In figure 6B, the tested compounds are related to the ZINC-NP reference 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.

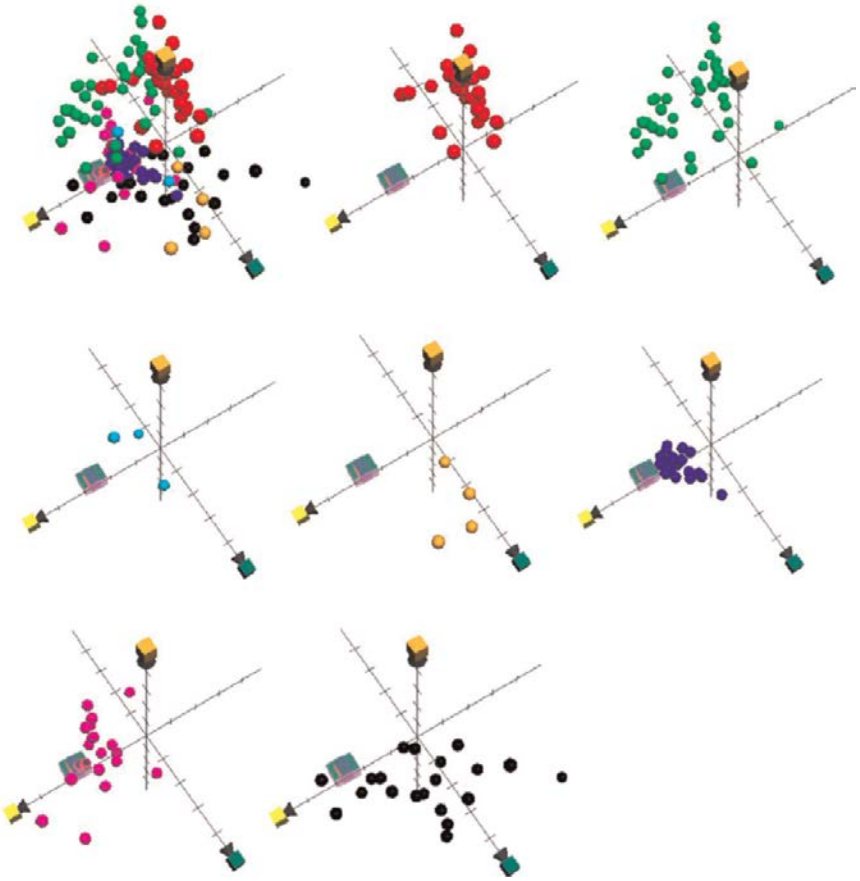

Figure 7. ChemGPS-NP analysis of calanquinone A and denbinobin. Score plot of the three dimensions (principal components 2–4) consisting of PC2 (yellow; aromaticity etc.), PC3 (green; lipophilicity etc.) and PC4 (orange; flexibility/rigidity), from analysis of most potent compounds 6a and 6b as medium 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 was 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.

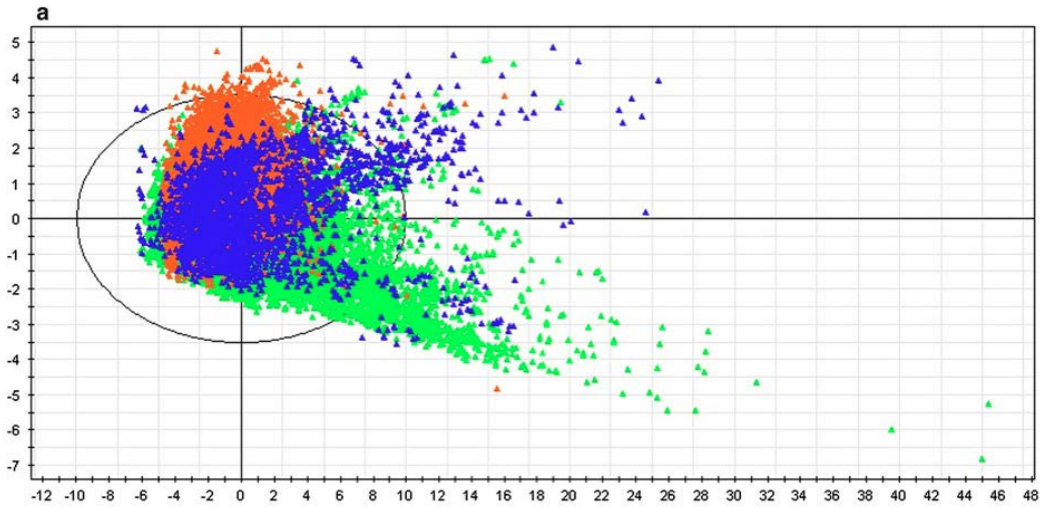

Figure 8. Results from chemographic mapping of marine (blue), terrestrial (green) and druglike (orange) compounds, compiled from literature. Plots clearly demonstrates differential coverage of multi-dimensional chemical property space. From Muigg *et al.*, 2012 in *Phytochemistry Reviews*.

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.

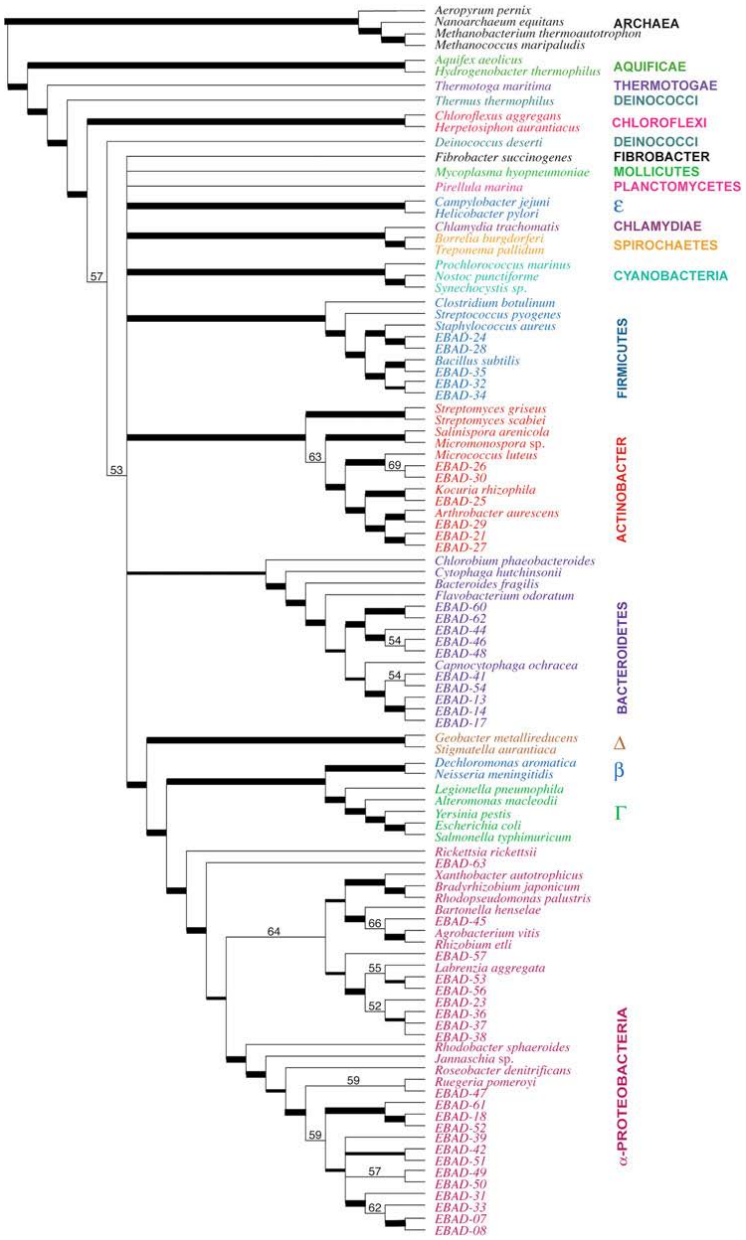

Figure 9. Phylogenetic tree a selected set of reference bacteria, and phylogenetic position of environmental samples from the interior of an *Acyonium* soft-coral (labels EBAD-#). This indicates not only that there is a wide diversity of bacteria living inside the coral, but also that these can be firmly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

During the fall of 2013 the group was visited by Dr. Tony Chen from National Museum of Marine Biology and Aquarium, Taiwan. Dr. Chen pursued an exploratory study on how to predict biological activity of previously unstudied natural compounds, using the ChemGPS-NP chemical property space. The visit was financed with support from Elisabeth & Alfred Ahlquist foundation.

Furthermore, the EU decided to support the ITN grant application "MedPlant". The ITN MedPlant is coordinated from University of Copenhagen, but includes one 'early stage researcher' (PhD-student) to be stationed at Uppsla Unievrsity under the supervision of Prof. Backlund, and a closely collaborating 'experienced researcher' (post-doc.) to be stationed at AstraZeneca R&D in Mölndal under the auspice of Dr. Thierry Kogej. The recruitment process of both positions was initiated during 2013.

Members of the group during 2013

Anders Backlund, Professor
Cecilia Alsmark, Assistant Professor
Christina Wedén, Postgraduate researcher, PhD
Sonny Larsson, Postgraduate researcher, PhD
Anna Koptina, Postgraduate researcher, PhD
Tony Chen, Postgraduate researcher, PhD
Elisabet Vikeved, MSc, PhD student
Åke Strese, MSc, PhD student

Publications 2011-2013

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. 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 – 2012

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5.

Reviews 2011-2013

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

Part of BlueGenics, to Uppsala totally (data from Lars Bohlin)
Elisabeth och Alfred Ahlquists undervisningsstiftelse
Part of MedPlant ITN, total budget ca €M 4,2, for Uppsala University together with partner Astra Zeneca € 486 697,20

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 2013

Lars Bohlin, Professor
Ulf Göransson, Associate 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.

Publications 2011-2013

1. M. Sjögren, P. Jonsson, M. Dahlström, T. Lundälv, R. Burman, U. Göransson, et al. Two brominated cyclic dipeptides released by the coldwater marine sponge Geodia barretti act in synergy as chemical defense. Journal of Natural Products. 2011; 74(3):449-454.

Agencies that support the work/Funding

VINNOVA 250 000 SEK/year
EU 700 000 SEK/year

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 2013

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

Publications 2011-2013

1. Ruhaak L, Felth J, Karlsson P, Rafter J, Verpoorte R, Bohlin L.
   Evaluation of the cyclooxygenase inhibiting effects of six major cannabinoids isolated from Cannabis sativa. Biological and Pharmaceutical Bulletin. 2011; 34(5):774-778.

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3. Hallböök H, Felth J, Eriksson A, Fryknäs M, Bohlin L, Larsson R, et al. Ex Vivo activity of cardiac glycosides in acute leukaemia. PLoS ONE. 2011; 6(1):e15718-.

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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 secretariat 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 mandatory courses in Computational Medicinal Chemistry and Drug Discovery and Development, 7,5 hp each, in cooperation with the two other Departments at the Faculty of Pharmacy. The focus of the programme is biomedical

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 2013

Professor Anders Hallberg, were named to receive honorary doctoral degrees from the Faculty of Pharmacy.

List of Staff

Department of Medicinal Chemistry

www.ilk.uu.se

Division of Analytical Pharmaceutical Chemistry, www.farmkemi.uu.se
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Matilda Lampinen Salomonsson
Alfred Svan
Eva Strömberg
Annica Tevell Åberg
Niklas Tyrefors
Douglas Westerlund

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Mathias Alterman
Per Arvidsson
Linda Axelsson
Anna-Karin Belfrage
Gunilla Eriksson
Ashkan Fardost
Rebecca Fransson
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