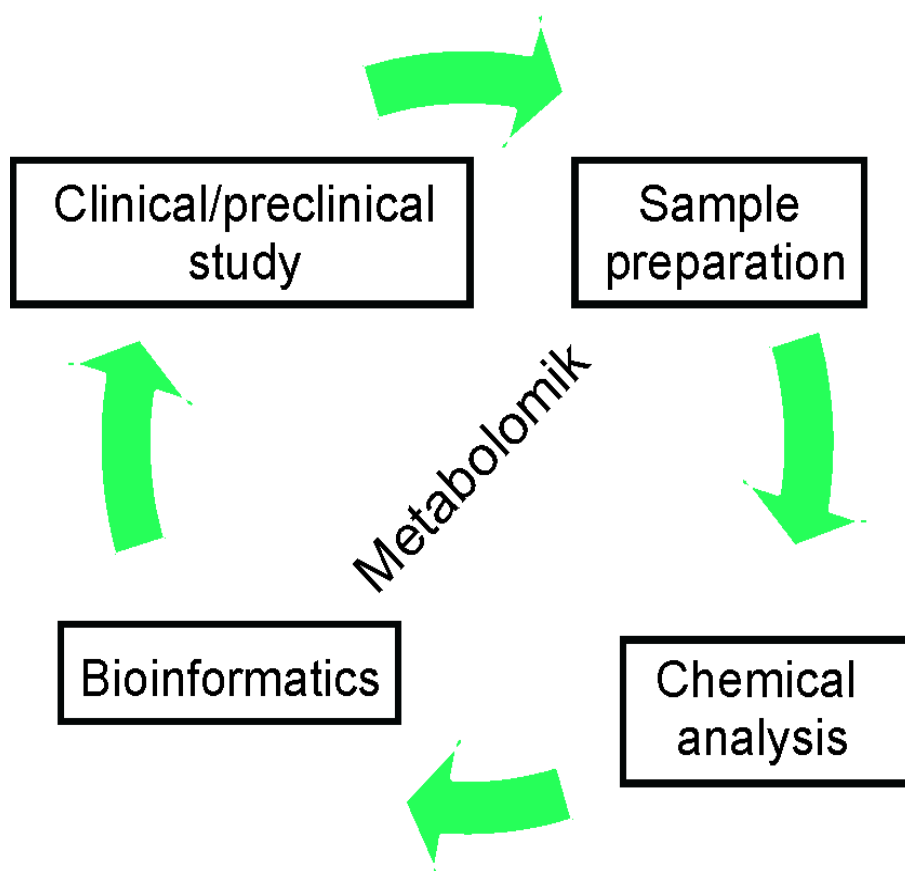




Annual Report 2012

Department of Medicinal Chemistry



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Front page illustration: Mikael Engskog

INTRODUCTION

The year 2012 has been a successful, inspiring and exciting year for the Department of Medicinal Chemistry, something which would not have been possible without our skilled researchers, teachers and administrators. The economic situation at the Department is sound and a small excess, less than the 10 % of the annual expenses which is the maximum allowed, has accumulated during the last years. Since the inflow of external grants is somewhat unpredictable, it is necessary to have a certain excess in order to fulfill the obligation in funding PhD students during their education as well as to enable investments in infrastructure essential for research and educational activities. As the research at the Department is strongly dependent on access to modern and efficient equipment, we are pleased that we have been able to make major investments in chromatographic systems (SFC, UPLC), state of the art mass spectrometry (Q-TOF, Synapt G2S), GC/MS systems as well as an advanced microscope and a multimode plate reader.

This annual report describes several projects in the main research areas: analytical pharmaceutical chemistry, computer-based drug design, medicinal chemistry, organic pharmaceutical chemistry, pharmacognosy, preclinical positron emission tomography (PET). Furthermore, the Division of Analytical Pharmaceutical Chemistry has engaged itself in a new exciting field, metabolomics, an interdisciplinary effort with collaborations at both Uppsala University and Karolinska Institute. All research projects are, and will in the future, be even more dependent on the ability to attract external grants. Senior and young researchers at the Department have devoted much time and effort in preparing research grant applications and are urged to continue with their great work. During 2012 Associate Professor Anna Orlova received a grant from the Swedish Research Council (Medicine and Health, 2700 kSEK) to support her research in "Improvement of diagnostics and personalised treatment of prostate cancer by radionuclide molecular imaging." and 1800 kSEK from the Swedish Cancer Society for her project "Radionuclide molecular imaging of prostate cancer: in vivo phenotyping of tumors for personalised medicine". Associate Professor Ulf Göransson also received support (1800 kSEK) from the Swedish Research Council (Natural and Engineering sciences) for the project Ultra Stable Protein-Based Drug Scaffolds. Researchers at the Division of Pharmacognosy are members of the research program BlueGenics that received 6 M€ within the EU FP7 in 2012. Despite the heavy work load, researchers at the Department are active in both national and international research networks. Professor Anders Backlund has since 2004 been collaborating in research and teaching with the Kaohsiung Medical University in Taiwan and was in 2012 awarded the title honorary visiting professor at that university. One of our young researchers, Dr. Rebecca Fransson, was awarded the Benzelius Prize by the Royal Society of Science in Uppsala for her thesis, 'Discovery of Small Peptides and Peptidomimetics Targeting the Substance P 1-7 Binding Site: Focus on Design, Synthesis, Structure-Activity Relationships and Drug-Like Properties'.

Professor Rob Verpoorte at the Natural Products Laboratory (Leiden University, Holland) has since the 1970's collaborated in research projects and teaching with the Division of Pharmacognosy. The Department highly appreciates Professor Verpoorte's contributions to the development of pharmacognosy in Sweden and he received a honorary doctorate at the Faculty of Pharmacy at the "vinterpromotionen" in 2012.

The government has given high priority to research in infectious diseases and antibiotic resistance, one of the main research areas at the Division of Organic Pharmaceutical Chemistry during the last 10 years. Professor. Mats Larhed was the chairman of the

organization committee for the SciLifeLab conference “In Joint Battle Against Infectious Disease and Antibiotic Resistance”, held the 23rd of November 2012 in Uppsala. This congress was a great success in its aim to initiate “A high-level debate where thought-provoking case studies of initiatives and innovations will act as catalysts for discussion between leading international biomedical researchers, industry leaders, policy-makers and healthcare experts”.

Teachers at our Department are very active in several of the undergraduate programs at the Faculty of Pharmacy and they are also engaged in courses at the Faculty of Medicine and Faculty of Science and Technology. The Department continues to focus on the implementation of computer-based exams (CBE) in our undergraduate programs. All teachers put in hard work to develop good courses, apply a qualified pedagogy and promote a friendly and good teaching environment for the students. In this context, I would like to congratulate Associate Professor Anja Sandström from the Division of Organic Pharmaceutical Chemistry, who received a scholarship from STINTs Program for Excellence in Teaching. Sandström will be visiting the Amherst College, Massachusetts, USA during the autumn of 2013.

Professor emeritus Gunnar Samuelsson who previously held the chair in Pharmacognosy became the first jubilee doctor at the Faculty of Pharmacy at the “vårpromotion” in 2012.

In May, professor Anders Hallberg, the former Head of the Department, Dean of the Faculty of Pharmacy, Deputy Vice President (Medicine/Pharmacy) and Vice Chancellor of Uppsala University retired. The Department organized a symposium dedicated to professor Hallberg on the 14th of June 2012. One of the speakers was the Nobel Prize laureate in chemistry Barry Sharpless. We are happy that Prof. Hallberg as emeritus will continue his research at the Department and hopefully he will participate in stimulating scientific discussions.

I would like to express my sincere thanks to all staff at the Department for their excellent work and commitment during 2012 and especially to Eva Rosendal Jansson and Håkan Hall for their important contributions and service during their time at the Department.

This Annual Report represents a brief summary of the activities of the Department of Medicinal Chemistry during 2012. 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.

Uppsala, April, 2013
Curt Pettersson, PhD, Professor
Head of the 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

Gunilla Eriksson, secretary, technical/administrative

Lars Bohlin, teacher representative

Mats Larhed, teacher representative

Anja Sandström, teacher representative

Albert Elmsjö, graduate student representative

Emilia Gjurovska, undergraduate student representative

Maj Blad, technical/administrative representative deputy

Ylva Hedeland, teacher representative, deputy

Anders Backlund, teacher representative, deputy

Professores emeriti

Anders Hallberg

Gunnar Samuelsson

Lars-Olof Sundelöf

Douglas Westerlund

Senior lecturer emeriti

Uno Svensson

Director of graduate studies

Anders Backlund

Secretariat

Gunilla Eriksson

Eva Strömberg

Course secretariat

Ann-Marie Benson

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

Anders Karlén (50%)

Anja Sandström (50%)

Preclinical 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

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 board of Uppsala University Center for Sustainable Development
- 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)
- Member of the Swedish Academy of Pharmaceutical Sciences
- Secretary of the scientific domain Faculty

Lars Bohlin

- Head of division of Pharmacognosy
- Vice-Chairman of the Phytochemical Society of Europe
- Evaluation expert for national research projects, Austria, 2011
- Opponent Univ. of Copenhagen, Denmark, 2011
- Member of the American Society of Pharmacognosy and of the American Botanical Council
- Co-editor of Phytochemistry Letters
- Chairman of Folkuniversitetet, Uppsala

Olof Eriksson

- Member of the European Association of Nuclear Medicine
- Member of Uppsala Medical Society
- Member of the European Association for the Study of Diabetes

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

Håkan Hall

- European College of Neuropsychopharmacology
- Association of European Psychiatrists.

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

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
- Director of undergraduate studies in organic pharmaceutical chemistry
- Chairman of the board of the Medicinal Chemistry Section of the Swedish Academy of Pharmaceutical Sciences
- 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 ULLA Executive Committee
- Member of the Editorial Board for ChemistryOPEN
- Member of the Royal Society of Sciences at Uppsala

Luke Odell

- Member of The Swedish Academy of Pharmaceutical Sciences
- Member of the Swedish Chemical Society
- Member of the Editorial Board for Current Microwave Chemistry
- Associate Editor; Science of Synthesis Cross-Coupling and Heck-Type Reactions, Volume 3, Metal-Catalyzed Heck-Type Reactions and C-C Cross Coupling via C–H Activation,

Anna Orlova

- Member of European Association of Nuclear Medicine
- Member of International Research group in Immuno-Scintigraphy and Therapy
- Member of the editorial board of International Journal of Organic Chemistry
- Member of the editorial board of Scientifica
- Member of the Technical Advisory Board of Affibody AB, Solna
- Responsible for animal experiments in ROS, Medical Faculty (Djurföreståndare)

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

Christian Sköld

- Member of the programme committee of the biomedicine programme, Uppsala University
- Chairman of the Committee for equality, Department of Medicinal Chemistry, Uppsala University

–

Ulrika Rosenström

- Member of The Swedish Academy of Pharmaceutical Sciences

Scientific reports

Analytical Pharmaceutical Chemistry

The research at the Division of Analytical Pharmaceutical Chemistry at the Department of Medicinal Chemistry is focused on separation science and mass spectrometry. The analytes of interest are drugs and their degradation products and metabolites as well as carbohydrates, peptides, proteins, amino acids and other small molecules.

The research is divided into two areas of importance: pharmaceutical analysis and bioanalysis. 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:

- Chiral separation methods
- Analysis of drugs in the environment
- High resolution NMR spectroscopy and UPLC-MS based metabolomics in pharmaceutical research

- Capillary electrophoresis for biomedical applications

Members of the group during 2012

Curt Pettersson, Professor
 Torbjörn Arvidsson, Associate Professor
 Mikael Engskog, PhD, Researcher
 Olle Gyllenhaal, Associate Professor
 Mikael Hedeland, Associate Professor
 Ylva Hedeland, PhD, Senior Lecturer
 Monika Johansson, Associate Professor
 Niklas Tyrefors, PhD, Researcher
 Victoria Barclay, PhD student
 Jakob Haglöf, PhD, Junior Lecturer
 Alexander Hellqvist, PhD student

Publications 2010-2012

1. O. Gyllenhaal, Karlsson A: Evaluation Conditions for SFC of Metoprolol and Related Amino Alcohols on Hypercarb (Porous Graphitic Carbon) with Respect to Structure–Selectivity Relations , [Chromatographia 71; 2010, 7-13](#)
2. J. Haglöf, Pettersson C: Separation of amino alcohols using divalent dipeptides as counter ions in aqueous CE, [Electrophoresis 31; 2010, 1706-1712](#)
3. T. Rundlöf, M. Mathiasson, S. Bekiroglu, B. Hakkarainen, T. Bowden, T. Arvidsson: Survey and qualification of internal standards for quantification by ¹H NMR spectroscopy, [J. Pharm. Biomed. Anal. 52; 2010, 645-651](#)
4. A. Persson, C. Pettersson, J. Rosén: Multivariate Data Analysis of Factors Affecting the *In Vitro* Dissolution Rate and the Apparent Solubility for a Model Basic Drug Substance in Aqueous Media, [Pharm. Res. 27; 2010, 1309-1317](#)
5. 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](#)
6. 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](#)
7. 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](#)

8. 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](#)

Reviews 2010 - 2012

Y. Hedeland, C. Pettersson Chapter 17: "HPLC applications for Chiral Pharmaceutical Analysis" in Handbook of HPLC Second Edition (Chromatographic Science series 101) Editors Danilo Corradini and Terry M. Phillips. *CRC-press* 2010. ISBN: 9781574445541

Y. Hedeland, C. Pettersson Chapter 10: "Chiral separations in non-aqueous media" in Chiral Separations by Capillary Electrophoresis (Chromatographic Science series 100). Editors A. van Eeckhaut och Y. Michotte. *CRC press* 2010. ISBN-13: 978-1-4200-6933-4

Dissertations 2012

Barclay, Victoria

Development of LC-MS/MS Methods for the Analysis of Chiral and Achiral Pharmaceuticals and Metabolites in Aqueous Environmental Matrices [Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 158, 2012](#)

Agencies that support the work/Funding

Medical Product Agency

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., β -adrenoceptor 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.

Analysis of drugs in the environment

Curt Pettersson, Monika Johansson, Niklas Tyrefors, Victoria Barclay, 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 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 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.

A chiral analytical method for the determination of fluoxetine and its metabolite norfluoxetine has been developed. Wastewater is extracted and concentrated with solid phase extraction and is further analysed with liquid chromatography coupled to tandem mass spectrometry. The chiral separation is performed with a chiral micro AGP column, and a mobile phase consisting of ammonium acetate buffer and acetonitrile. The analytes are detected in positive ion mode and by selected reaction monitoring. The enantiomers of fluoxetine and norfluoxetine were quantified in the wastewater samples and the concentrations of the enantiomers were determined in both raw and treated wastewater. Furthermore, the method was validated, in the wastewater in which the compounds were detected, by the use of the stable isotope-labelled compounds fluoxetine- $^2\text{H}_5$ and norfluoxetine- $^2\text{H}_5$.

A chiral LC-MS/MS method has also been developed for the quantification of metoprolol and two of its chiral human metabolites. The water samples are enriched by the use of solid phase extraction and the stereoisomers are separated using two different protein based chiral stationary phases. The target compounds are detected by tandem quadrupole mass spectrometry. The method was employed for the determination of the stereoisomers of the target compounds in treated wastewater samples from a wastewater treatment plant in Uppsala. Six of the eight stereoisomers were quantified in the wastewater that was discharged from the wastewater treatment plant into the River Fyris in Uppsala. The method can be used to get better insights about the chiral degradation of these compounds in aqueous environmental matrices.

A method for achiral determination of the benzodiazepine diazepam and its metabolite nordiazepam in wastewater has also been developed within the project. Using octadecyl extraction discs, influent and effluent wastewater was successfully extracted in such way that the risk of sample contamination was minimised. Diazepam and nordiazepam were completely separated within 3.5 minutes with a Pro C₁₈ column from YMC and a C₈ SecurityGuard from Phenomenex®. Gradient elution is performed with formic acid in water and formic acid in acetonitrile. Isotope-labelled internal standards, diazepam-²H₅ and nordiazepam-²H₅, were used to compensate for expected ion suppression and matrix effects. The analytes are detected with tandem mass spectrometry in positive ion mode by use of selective reaction monitoring to achieve the required selectivity and sensitivity.

The two ongoing projects are focusing on metoprolol; one for determination of its degradation in Californian wetlands and one for evaluation of a new solid phase extraction method.

So far, the project has led to three publications; two from the work with fluoxetine and its metabolite norfluoxetine and one from the work with metoprolol and two of its human metabolites.

High resolution NMR spectroscopy and UPLC-MS methodology development for metabolomics investigations

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

This multidisciplinary project aims to develop, establish and validate an analytical methodology for metabolomics investigations as well as to apply this platform in a diverse set of pilot-projects. We aim to find scientifically reliable analytical systems for detection, identification and quantification of metabolites in biological samples such as plasma and cells. The literature dealing with metabolomics have grown tremendously during the last five years, though little 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 metabolic fingerprints, (iii) automatic detection of signal by mass spectrometry (MS) and/or nuclear magnetic resonance (NMR) spectroscopy, (iv) statistical analysis and, ultimately, (v) identification of altered pathways. Recent publications have concluded that a combined use of NMR spectroscopy and MS provide beneficial synergies for metabolomics purposes. This approach will be used throughout the projects as the department and included collaborators have extensive theoretical and practical knowledge of both systems as well as access to them. For statistical analysis, two key areas have been identified: data dimensionality reduction tools, like Principal Component Analysis (PCA), focus on data variability and aid the identification of interesting parts of the NMR and MS spectra;

Discriminant Analysis (DA), particularly in combination with Orthogonal Projection to Latent Structures (OPLS), provide a means to pinpoint differences between groups of samples.

The analytical methodology is being developed and evaluated in several interesting ongoing studies through established collaborators found locally in Uppsala. Two applications are briefly introduced below.

Within the field of toxicology the non-protein amino acid β -methylamino-L-alanine (BMAA) is studied as a potential human neurotoxin with a strong connection to the neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS). BMAA is produced by most cyanobacteria and detected in strains found in Swedish lakes and the Baltic Sea and correlates with increased levels of the amino acids glutamate, aspartate and alanine in the central nervous system. The project aims to clarify the mechanism for the observed cognitive disturbance and morphological brain changes in rodents neonatally exposed to BMAA. Rodents have been treated neonatally after which serum are studied with the help of metabolomics to relate exposure of BMAA to possible neurotoxicological changes (Collaboration with Eva Brittebo, *Division of Toxicology, Department of Pharmaceutical Biosciences*).

The cancer pharmacology efforts are based on already ongoing pre-clinical pharmacology research being performed at the Department of Medical Sciences. In this work, metabolic profiles representing drug responses in different normal and cancer cell lines are studied to characterize how different drug families are altering metabolite profiles. This will result in unique information about how drugs can alter the metabolic activities in cancer and normal cells and how the response pattern can be related to anti-cancer drug resistance in the clinic, one of the most outstanding difficulties in current cancer therapy. If successfully completed, this effort will be an important step towards cost-effective individualized selection of cancer chemotherapy and it will also contribute to new drug discoveries and developments via the identification of drug induced metabolic changes which differ significantly between normal and cancer cells (Collaboration with Mats Gustafsson, Medical Bioinformatics, Department of Medical Sciences and Ulf Hammerling, National Food Administration).

Selected publications:

A NMR-based metabolomics study of short-term effects of neonatal exposure to β -methylamino-L-alanine (BMAA) in rats (*in manuscript*)

Mikael K. R. Engskog, Jakob Haglöf, Oskar Karlsson, Albert Elmsjö, Eva Brittebo, Torbjörn Arvidsson and Curt Pettersson

Optimized sample preparation procedures for NMR-based metabolomics of in vitro cancer cells (*in manuscript*)

Jakob Haglöf, Mikael K. R. Engskog, Obaid Aftab, Albert Elmsjö, Ulf Hammerling, Mats Gustafsson, Torbjörn Arvidsson, Curt Pettersson

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).

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 iothexol, 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.

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

Research Group Leader: Ulf Bondesson

Liquid chromatography - tandem mass spectrometry (LC-MS/MS) has become the most powerful technique for low-level determinations of drugs and their metabolites in biological fluids. As drug metabolites may be more active than the parent compound, or even toxic, it is of 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 2012

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

Reviews 2010-2012

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Dissertations 2012

-

Agencies that support the work/Funding

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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 Leaders: 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

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

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

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

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

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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. $-\text{CH}(\text{OH})\text{CH}_2\text{NH}-$) 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 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

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, Peter Nilsson, 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 scope 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 2012

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 Patrik Nordeman, PhD student
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 Jean-Baptiste Veron, PostDoc
 Peter Nilsson, Guest Researcher
 Jonas Rydfjord, PhD student
 Charlotta Wallinder, Research Associate
 Hitesh Motwani, PostDoc
 Linda Åkerbladh, PhD student
 Marc Stevens, PhD student
 Fredrik Svensson, PhD student
 Maria de Rosa, Post Doc
 Rajendra Mane, Post Doc

Publications 2010 - 2012

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3. Gising, Johan (Uppsala University, Disciplinary Domain of Medicine and Pharmacy, Faculty of Pharmacy, Department of Medicinal Chemistry, Organic Pharmaceutical Chemistry). Design and Synthesis of Enzyme Inhibitors Against Infectious Diseases: Targeting Hepatitis C Virus NS3 Protease and *Mycobacterium tuberculosis* Ribonucleotide Reductase. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 160

Agencies that support the work/Funding

1. Swedish Research Council, 900 000 SEK for 2012
2. Vinnova, 1 100 000 SEK for 2012

3. Swedish Academy of Pharmaceutical Sciences, 250 000 SEK for 2012
4. Wavecraft

Heterocyclic Chemistry

Luke Odell, Marc Stevens, Krzysztof Wieckowski, Peng Wu, Mattieu Desroses

Background: Heterocycles are of extreme importance in drug discovery and medicinal chemistry. The vast majority of marketed drugs are either heterocycles or contain heterocyclic fragments. Thus, new methodologies for the construction or functionalization of heterocyclic 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 methodology.

Aim: To develop new methods for the synthesis and functionalization of heterocyclic ring systems. To utilize these methods in the preparation of biologically active compounds.

Method: We have recently reported a novel base catalyzed method for the synthesis of 1N-hydroxyindazoles, from 2-nitrobenzylamine derivatives. During the course of this investigation, a novel one-pot cyclization/etherification reaction was discovered. We plan to optimize and explore the scope of this novel transformation, which should lead to a powerful new strategy for the synthesis of highly substituted indazoles. In addition, we will also investigate methodology for the functionalization of indazoles and indoles via a C-H activation approach as well as various other novel ring synthesis methods.

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3. M. Desroses, K. Wieckowski, M. Stevens, L.R Odell: A microwave-assisted, propylphosphonic anhydride (T3P®) mediated one-pot Fischer indole synthesis. *Tetrahedron Lett.* 2011, 52, 4417-4420

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. We recently characterized the important role of benzoquinone in the diaryl addition reaction of a chelating olefin and we are currently investigating ligand and solvent effects for Pd(II)-catalyzed decarboxylative addition to nitriles.

Publications 2010–2012

1. C. Sköld, J. Kleimark, A. Trejos, L. R. Odell, S. O. Nilsson Lill, P.-O. Norrby, M. Larhed: Transmetalation 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.

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, Johan Åqvist, 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 2012

Anders Karlén, Professor
 Mats Larhed, Professor
 Johan Gising, PhD Student
 Hiba Alogheli, PhD Student
 Linda Åkerbladh, PhD Student
 Martin Lindh, PhD Student
 Bobo Skillinghaug, PhD Student
 Fredrik Svensson, PhD Student
 Shyamraj Dharavath, Postdoctoral Fellow
 Rajendra Mane, Postdoctoral Fellow
 Hitesh Motwani, Postdoctoral Fellow
 Luke Odell, Research Associate
 Christian Sköld, Research Associate
 Gunnar Lindeberg, Research Associate

Publications 2009 - 2011

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Agencies that support the work

1. 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

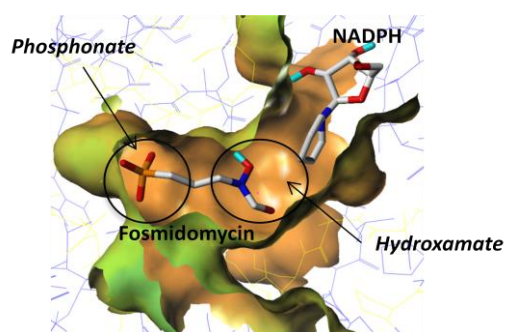
Ribonucleotide reductase (RNR) catalyses the reduction of ribonucleotides to the corresponding deoxyribonucleotides and is an essential enzyme for DNA synthesis. The active enzyme is a tetramer composed of two large subunits (R1) and two small subunits (R2). R1 possesses the substrate and effector binding 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.

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

Publications 2010-2012

1. P. Örtqvist, J. Gising, A. E. Ehrenberg, A. Vema, A. Borg, A. Karlén, M. Larhed, U. H. Danielson, A. Sandström: Discovery of achiral inhibitors of the Hepatitis C virus NS3 protease based on 2(1H)-pyrazinones. *Bioorg. Med. Chem.*; 18; 2010, 6512-6525
2. H. Andersson, H. Demagdt, G. Vauquelin, G. Lindeberg, A. Karlen, M. Hallberg, M. Erdelyi, A. Hallberg: Disulfide Cyclized Tripeptide Analogues of Angiotensin IV as Potent and Selective Inhibitors of Insulin-Regulated Amino peptidase (IRAP). *J. Med. Chem.* 53; 2010, 8059-8071.
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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 mammals 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 2012

Anja Sandström, Associate Professor
Eva Åkerblom, Associate Professor
Anders Karlén, Professor

Mats Larhed, Professor
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 Rebecca Fransson, PhD
 Anna Lampa, PhD student
 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 2010-2012

1. R. Fransson, M. Botros, C. Sköld, F. Nyberg, G. Lindeberg, M. Hallberg, A. Sandström: Discovery of Dipeptides with High Affinity to the Specific Binding Site for Substance P₁₋₇. *J. Med. Chem.* 53; 2010, 2383–2389.
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3. A. Lampa, A. E. Ehrenberg, S. S. Gustafsson, A. Vema, E. Åkerblom, G. Lindeberg, A. Karlén, U. H. Danielson, A. Sandström: Improved P2 Phenylglycine-based Hepatitis C Virus NS3 Protease Inhibitors with Alkenylic Primeside Substituents. *Bioorg. Med. Chem.* 18; 2010, 5413-5424.
4. P. Örtqvist, J. Gising, A. E. Ehrenberg, A. Vema, A. Borg, A. Karlén, M. Larhed, U. H. Danielson, A. Sandström: Discovery of achiral inhibitors of the Hepatitis C virus NS3 protease based on 2(1H)-pyrazinones. *Bioorg. Med. Chem.* 18; 2010, 6512-6525.
5. 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. *Peptides*. 2011, 32, 93-98.
6. A. Lampa, A. E. Ehrenberg, A. Vema, E. Åkerblom, G. Lindeberg, U.H. Danielson, A. Karlén, A. Sandström: P2-P1' macrocyclization of P2 phenylglycine based HCV NS3 protease inhibitors using ring-closing metathesis. *Bioorg. Med. Chem.* 2011, 19, 4917-4927.
7. M. Ohsawa, A. Carlsson, M. Asato, T. Koizumi, Y. Nakanishi, R. Fransson, A. Sandström, M. Hallberg, F. Nyberg, J. Kamei: The dipeptide Phe-Phe amide attenuates signs of hyperalgesia, allodynia and nociception in diabetic mice using a mechanism involving the sigma receptor system. *Molecular Pain*. 2011, 7:85.

8. S. Clearhout, S. Sharma, C. Sköld, C. Cavaluzzo, A. Sandström, M. Larhed, M. Thirumal, V. S. Parmar, E. V. Van der Eycken. Synthesis of Functionalized Europyrazines as Restricted Dipeptidomimetics. *Tetrahedron* (2012), 68, 3019-3029
9. R. Fransson, A. Sandström. Use of Peptides as Drug Leads—A Case Story on the Development of Dipeptides Corresponding to the Heptapeptide Substance P(1-7), with Intriguing Effects on Neuropathic Pain. In *Neuropeptides in Neuroprotection and Neuroregeneration*. F. Nyberg, ed.; CRC press Taylor & Francis Group.; Boca Raton, 2012; pp 253-270.

Dissertations 2012:

10. Lampa, Anna. Design and Synthesis of Acyclic and Macrocyclic Peptidomimetics as Inhibitors of the Hepatitis C Virus NS3 Protease. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 152

The Hepatitis C Project

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

Hepatitis C virus (HCV) is recognized as a major cause of end-stage liver disease as well as the leading cause of liver transplantations in the developed world. It is estimated that 2–3% of the world population is infected with this blood-borne pathogen. The development of safer and more efficient anti-HCV drugs, especially those targeting the virus directly, is highly desirable.

Viral proteases are considered attractive antiviral targets mainly as a result of the successful use of HIV-1 protease inhibitors that revolutionized the HIV/AIDS therapy. Consequently, the HCV NS3 protease is one of the most intensively studied anti-HCV targets and most well-characterized HCV enzymes. In its native form the NS3 protease is covalently attached to another enzyme, the NS3 helicase/NTPase, and the total is designated the full-length NS3 protein. HCV protease inhibitors are nowadays verified antiviral agents, due to two recently approved drugs in this class. There are vast amounts of potent inhibitors under development, including those on the market that are based on a peptidic backbone encompassing a P2 proline-like moiety. However, structural similarities convey issues with cross-resistance, underlining the need for novel drugs with unique resistance profiles, beside those of different mechanisms of actions, which can be used in a future combination therapy to combat HCV.

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 focusing on a continued optimization of our protease inhibitors into peptide mimetics with an overall attractive pharmacokinetic drug profile and with unique structure elements, providing distinct resistance profiles compared to other inhibitors encompassing P2 proline

residues. This work includes evaluation of a non-peptidic beta-sheet mimetic in the P3-P2 region of the inhibitors, new P2 scaffolds that allow substitution of various functional groups by different chemical transformation, and a non-peptidic aromatic P1-residue. Both acyclic and macrocyclic inhibitors are produced. Moreover, we are scrutinizing the promising acyl sulfonamide group and developing bioisosteres thereof, to guide further improvements of this crucial C-terminal group interacting with the active site of the protease.

The Neuropeptide Project

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

Substances that act as or block the effects of certain neuropeptides may constitute future therapeutics for neurological diseases such as drug dependence, memory disorders, or chronic neuropathic pain. The specific aims of this project are to design and develop stable and bioavailable peptidomimetics of the neuropeptide Substance P 1-7 (SP₁₋₇) that can be used as a research tool in functional animal studies for a more thorough understanding of the physiological function of SP₁₋₇, including identification of its macromolecular target.

Substance P 1-7 (SP₁₋₇ = 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 SP₁₋₇ at a molecular level, including receptor recognition, are still unclear. However, Prof. Nyberg and Doc. Mathias Hallberg have identified specific binding sites for SP₁₋₇ in the rat spinal cord. Even though the intriguing effects of SP₁₋₇ have been known for quite some time SP₁₋₇ has not previously been addressed in a medicinal chemistry program. Our initial efforts in this area included a thorough SAR study of the binding of SP₁₋₇ and endomorphin-2 (EM-2) to the SP₁₋₇-binding site by means of Ala-scans, truncation studies and C- and N-terminal modifications of the two target peptides. From this we concluded that only the C-terminal part, and in particular the C-terminal phenylalanine, were crucial for the affinity. Moreover, C-terminal amidation potentiated the ligands five-fold; an effect which was also reproduced in an *in vivo* study where the amidated analogue of SP₁₋₇ was shown to reduce opioid withdrawal signs in rats more effectively than the native heptapeptide. Altogether, the SAR studies led to the remarkable discovery of the dipeptide H-Phe-Phe-NH₂ ($K_i = 1.5$ nM), having equal affinity as endogenous heptapeptide ligand SP₁₋₇ and consequently higher affinity than the mother peptide EM-2. SP₁₋₇, SP₁₋₇-amide and H-Phe-Phe-NH₂ were further evaluated for their potential antinociceptive effect in non-diabetic mice and diabetic mice after intrathecal administration. All compounds, including the small dipeptide, showed a concentration dependent antinociceptive effect in both mice models. Interestingly, the effect was higher in diabetic mice, which suggest that the compounds are more effective against pain of neuropathic origin. 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, are currently under preparation in order to improve the pharmacokinetics properties of the ligands.

Publications from Division members in 2010 – 2012, unrelated to the projects above

1. M. M. Makatini, K. Petzold, S. N. Sriharsha, M. E. S. Soliman, B. Honarparvar, P. I. Arvidsson, Y. Sayed, P. Govender, G. E. M. Maguire, H. G. Kruger, T. Govender: Pentacycloundecane-based inhibitors of wild-type C-South African HIV-protease. *Bioorg. Med. Chem. Lett.* 2011, *21*, 2274-2277.
2. M. M. Makatini, K. Petzold, S. N. Sriharsha, N. Ndlovu, M. E. S. Soliman, B. Honarparvar, R. Parboosing, A. Naidoo, P. I. Arvidsson, Y. Sayed, P. Govender, G. E. M. Maguire, H. G. Kruger, T. Govender: Synthesis and structural studies of pentacycloundecane-based HIV-1 PR inhibitors: A hybrid 2D NMR and docking/QM/MM/MD approach. *Eur. J. Med. Chem.* 2011, *46*, 3976-3985.
3. T. Naicker, K. Petzold, T. Singh, P. I. Arvidsson, H. G. Kruger, G. E. M. Maguire, T. Govender: Novel tetrahydroisoquinoline based organocatalysts for asymmetric Diels-Alder reactions: insight into the catalytic mode using ROESY NMR and DFT studies. *Tetrahedron: Asymmetry* 2010, *21*, 2859-2867.
4. T. Naicker, P. I. Arvidsson, H. G. Kruger, G. E. M. Maguire, T. Govender: Tetrahydro-isoquinoline-Based *N*-Oxides as Chiral Organocatalysts for the Asymmetric Allylation of Aldehydes. *Eur. J. Org. Chem.* 2011, *34*, 6923-6932.
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6. F. Sehgelmedble, J. Jansson, C. Ray, S. Rosqvist, S. Gustavsson, L. I. Nilsson, A. Minidis, J. Holenz, D. Rotticci, J. Lundkvist, P. I. Arvidsson*: Sulfonimidamides as Sulfonamides Bioisosteres: Rational Evaluation through Synthetic, in Vitro, and in Vivo Studies with γ -Secretase Inhibitors. *ChemMedChem*, *7* (2012) 396-399.
7. M. F. Maldonado, F. Sehgelmedble, F. Bjarnemark, M. Svensson, J. Åhman, P. I. Arvidsson*: Synthesis and arylation of unprotected sulfonimidamides. *Tetrahedron* 68 (2012), 7456-7462. DOI: 10.1016/j.tet.2012.06.072
8. I. Mascari, Y. Besidski, G. Csajenyik, L. I. Nilsson, L. Sandberg, U. Yngve, K. Åhlin, T. Bueters, A. B. Eriksson, P.-E. Lund, E. Venyike, S. Oerther, K. Hygge Blakeman, L. Luo, P. I. Arvidsson*: 3-Oxoisoindoline-1-carboxamides: Potent, State-Dependent Blockers of Voltage-Gated Sodium Channel Nav1.7 with Efficacy in Rat Pain Models. *J. Med. Chem.* 55 (2012), 6866-6880. DOI: 10.1021/jm300623u
9. T. Naicker, P. I. Arvidsson, H. G. Kruger, G. E. M. Maguire, T. Govender*: Microwave-Assisted synthesis of Guandidin Organocatalysts Bearing a Tetrahydroisopuinline Framework and Their Evaluation in Michael Addition Reactions. *Eur. J. Org. Chem.* (2012), 3331-3337. DOI: 10.1002/ejoc.201200303

10. N. M. Hemmaragala, P. I. Arvidsson, G. E. M. Maguire, H. G Kruger, T. Govander*:
Interaction of β -Amyloid Interactions with Peptide Functionalized Gold Nanoparticles
J. Nanosci. Nanotechnol. 12 (2012) 2179-2184. DOI: 10.1166/jnn.2012.5791

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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

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 2012.

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.
 - Neuroendocrine tumours studied with a SV2A selective PET tracer
 - Autoradiography study of angiogenesis in abdominal aortic aneurysm with [¹⁸F]fluciclatide – an $\alpha_v\beta_3$ integrin ligand
 - Synthesis and preclinical evaluation of a ¹¹C-labelled libiguin - searching for a new brain receptor potentially involved in the regulation of sexual behaviors
 - Characterization of binding of [¹¹C]D-deprenyl to animal inflammation models
- Neurodegeneration and other brain disorders
 - *In vitro* studies of central and systemic and A β -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 ¹¹C and ¹⁸F- 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 ¹¹CO

Members of PPP in 2012

Gunnar Antoni, Associate Professor
 Veronika Asplund, Research Engineer
 Sara Bergman, PhD student
 Jonas Eriksson, Scientist
 Olof Eriksson, Postdoctoral fellow
 Sergio Estrada, Scientist
 Ola Åberg, Scientist
 Håkan Hall, Adjunct Professor
 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
 Marie Svedberg, Scientist
 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 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 is most likely long 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. Clearly, a novel non-invasive methodology for *in vivo* quantification of islet mass would provide several advantages compared to current standard techniques.

Radiological modalities such as PET and to a lesser extent 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 enormous significance not only in relation to type 1 diabetes (T1D), but to type 2 diabetes (T2D). The change in islet mass in 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 addition, we identify 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 controls and several models of diabetes by means of a clinical PET/CT scanner, 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 2012

Olof Eriksson, Postdoctoral fellow
 Ramkumar Selvaraju, PhD student
 Irina Velikyan, Associate Professor
 Ewa Hellström-Lindahl, Associate Professor
 Ola Åberg, Scientist

Funding

1. Barndiabetesfonden, 2011-2012
2. ExoDiab 2012
3. Tore Nilssons Stiftelse 2012
4. Vinnova 2010-2012
5. JDRF, 2010-2012
6. EFSD 2012

Publications 2010 – 2012

1. O. Eriksson, M. Jahan, P. Johnström, O. Korsgren, A. Sundin, C. Halldin, L. Johansson. In vivo and in vitro characterization of [^{18}F]-FE-(+)-DTBZ as a tracer for beta-cell mass. *Nucl Med Biol* 37; 2010, 357-363.
2. 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.
3. O. Eriksson, B. Långström, R. Josephsson. Assessment of receptor occupancy-over-time of two dopamine transporter inhibitors by [^{11}C]CIT and target controlled infusion. *Uppsala J Med Sci* 116; 2011, 100-106.
4. 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.

5. 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.

Reviews 2010 – 2012

1. O. Eriksson, A. Alavi. Imaging the islet graft by Positron Emission Tomography. *Eur J Nucl Med Mol Imaging*. 2012 Mar;39(3):533-42.
2. D. Espes, O. Eriksson, J. Lau, P.O. Carlsson. Striated muscle as implantation site for transplanted pancreatic islets. *J Transplant* 2011; 2011, 352043.

Molecular imaging tracer development

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

Research Group Leader: Mats Larhed

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

The aim of this project is to design, synthesize and evaluate new selective nonpeptidic AT2 receptor PET tracers. We have established relevant AT1 and AT2 receptor assays that allow fast and efficient screening. A selective AT2 receptor agonist is used as the starting point for the development and our strategy involves systematic modifications of tracer candidates and radiolabelling with ^{11}C . 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 2012

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

Publications 2010 – 2012

1. O. Åberg, M. Stevens, J. Lindh, C. Wallinder, H. Hall, A. Monazzam, M. Larhed, B. Långström. Synthesis and evaluation of a ^{11}C -labelled angiotensin II AT2 receptor ligand. *J. Label Compd. Radiopharm*, 53; 2010, 616-624.

Development of 5-Fluoro- $[\beta\text{-}^{11}\text{C}]$ -L-tryptophan as a functional analogue of 5-hydroxy- $[\beta\text{-}^{11}\text{C}]$ -L-tryptophan for PET studies of neuroendocrine tumours and β -cell mass in pancreas

Research Group Leader: Gunnar Antoni and Olof Eriksson

5-Hydroxy- $[\beta\text{-}^{11}\text{C}]$ -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- $[\beta\text{-}^{11}\text{C}]$ -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 2012

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- $[\beta\text{-}^{11}\text{C}]$ -L-tryptophan is a functional analogue of 5-hydroxy- $[\beta\text{-}^{11}\text{C}]$ -L-tryptophan in vitro but not in vivo. Accepted for publication in *Nuc Med Biol Jan 2013*

Development of PET tracers for the study of fibrosis**Research Group Leader: Gunnar Antoni**

Fibrosis is characterized by an increase and pathologic accumulation of collagen, a major constituent of the extracellular matrix. The main constituents in fibrosis are collagen type I and type II, forming fibrils composed of three α chains. The increase in collagen content found in fibrotic tissue is also combined with a remodelling process where the fibers are more

cross-linked and aligned in one direction compared to normal extracellular matrix having a typical random direction of the collagen fibrils. This mechanically changes the properties of the tissue that becomes stiff. An increase in the ratio of collagen I to collagen II is also seen. In many chronic diseases fibrosis gives an important contribution to the symptoms and it is estimated that in USA up to 45% of all deaths can be related to disease involving fibrosis. All major organs can be affected by fibrosis with lungs, kidney and liver as particularly sensitive. In idiopathic pulmonary fibrosis 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 with NOTA and other chelates and labelled with ^{68}Ga . This also gives the opportunity to label with ^{18}F in the form of FAI^{2+} . The labelled peptides will be evaluated in *in vitro* assays and *in vivo* using small animal PET. Biopsis of fibrotic tissue from patients will also be used to characterize the tracer candidates. The lipophilicity of the tracers can also be modified with pegylation giving the option of directing the excretion to either a renal (hydrophilic) or hepatic (lipophilic) 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.

Members of the group during 2012

Gunnar Antoni, Associate Professor
 Olof Eriksson, Postdoctoral fellow
 Gunnar Lindeberg, Research Associate
 Ulrika Rosenström, Guest lecturer
 Irina Velikyan, Associate Professor

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 2012

Gunnar Antoni, Associate Professor
Veronika Asplund, Research Engineer

Neuroendocrine tumours studied with a SV2A selective PET tracer

Research Group Leaders: Gunnar Antoni and Sergio Estrada

The synaptic vesicle protein SV2A has been identified as a novel transmembrane transporter of neurotransmitters. It has also been shown to be a neuroendocrine cell marker and immunohistochemical stainings indicate that the immunoreactivity for SV2A-protein and analogs is as strong as or stronger than for chromogranin A in neuroendocrine tumors. Some years ago we tested if BON tumor cells, an established neuroendocrine tumor cell line, expressed the SV2A-protein, and they do. Other cell lines, e.g. KRJ-1, are now available that could also be tested. BON tumor cell xenografts in mice have been used for *in vivo* animal studies.

The fact, that neuroendocrine tumor cells contain the SV2A-protein and that there now might be a useful PET-tracer available that can bind to the SV2A-protein, make these tracers very interesting for *in vivo* PET imaging in neuroendocrine tumors. Localization and staging of these tumors is challenging for several reasons:

- Some tumors are small and can't be detected by conventional methods (CT, magnetic resonance imaging (MRI), ultrasound (US))
- Most tumors have metastasized to lymph nodes, liver, or other distant sites, and the extent needs to be established before therapeutic decisions
- After surgery, residual disease or recurrences need to be diagnosed,
- Treatment effects should be monitored. PET-imaging with [^{11}C]5-HTP, [^{18}F]DOPA and [^{68}Ga]DOTATOC is very promising and more informative than conventional methods but none of these modalities visualizes 100% of neuroendocrine tumors

Because of the universal expression of the SV2A-protein in neuroendocrine tumors, the new SV2A-binding PET-tracer would be very interesting. However, preclinical testing *in vitro* and *in vivo* in animals should be done first. Quantification of the expression of SV2A in endocrine tumours and study its relation to tumour growth, serotonin production and other downstream markers of tumour specific processes may be of clinical value and could thus give an insight into tumour biology and eventually give rise to new treatment paradigms.

Members of the group during 2012

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Synthesis and preclinical evaluation of a ^{11}C -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 2012

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Autoradiography study of angiogenesis in abdominal aortic aneurysm with [^{18}F]fluciclatide – an $\alpha_v\beta_3$ 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 2012

Gunnar Antoni, Associate Professor

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Characterization of binding of [¹¹C]D-deprenyl to animal inflammation models

Research Group Leader: Håkan Hall

Visualization of peripheral pain related to inflammatory processes is of great importance for the development of treatment for the individual patient. Previously, scientists collaborating in this project have found markers that distinctly accumulate in painful areas in patients suffering from chronic whiplash-associated disorders and following wrist distortions. Using a PET camera designed for small animals it is possible to further study the visualization of the inflammation associated to pain. In this project have selected some compounds known to accumulate in inflammation. The compounds were labelled with the positron emitter ¹¹C and characterized in rat models for inflammatory and post-operative (incisional) pain.

L-Deprenyl binds to the monoamine oxidase B (MAO-B) enzyme, which is increased in astrocytes, and is involved in the neuroinflammatory process. In the present investigation we have used [¹¹C]D-deprenyl, where the mechanism of action is unclear, but which can be used as a radiotracer for the visualization of pain in connection to neuroinflammation. In the study we used [¹¹C]L-dideuterated deprenyl and [¹⁸F]fluoro deoxy glucose in parallel to [¹¹C]D-deprenyl in similarly treated animals. Furthermore, radiolabeled analogues to deprenyl will be studied using *in vitro* autoradiography using tissue obtained during surgery.

This project is performed in close collaboration with scientists at Uppsala University Hospital.

Members of the group during 2012

Håkan Hall, Adjunct Professor

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Neurodegeneration and other brain disorders

***In vitro* studies of central and systemic and A β -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 β). 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 A β -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 A β 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 A β and binding of both ^{11}C - and ^3H -labelled PIB.

In the present project we have characterized [^3H]PIB binding *in vitro* to different tissues involved in systemic amyloidosis in comparison to AD brain. *In vitro* binding studies were conducted using [^3H]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 [^3H]PIB was observed in all tissues from patients with systemic amyloidosis. The mean value of [^3H]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 [^3H]PIB were observed but much less frequent in tissues from patients with systemic amyloidosis.

Members of the group during 2012

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Design and synthesis of a PET tracer for the study of the Vesicular Acetylcholine Transporter (VACHT)

Research Group Leader: Gunnar Antoni

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. AD in particular is characterised by cognitive impairment and is today the most common cause for dementia. Due to the aging population AD 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 with PET is today the main modality for gaining insight into neurotransmission in the living brain. Above all, PET provides the tools for the study 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.

The Vesicular Acetylcholine Transporter (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 in for example 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 ($T_{1/2} = 20.4$ min) and potentially also fluorine-18 ($T_{1/2} = 109$ 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.

Members of the group during 2012

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

Research Group Leaders: Mats Larhed, 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 A β 42, 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 A β 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 2012

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

Development of an antibody-based PET radioligand for Alzheimer's disease

Research Group Leader: Håkan Hall

There is a great need to reliably diagnose AD at an early stage and to monitor disease progression in the brain. Amyloid- β (A β) is a 40-42 amino acid long hydrophobic and self-aggregating peptide, which is central to AD pathogenesis. A β monomers gradually aggregate into soluble oligomeric assemblies and eventually into insoluble fibrils. A β fibrils, the main constituents of senile plaques which are a hallmark of AD, have a cross- β structure of the

peptide chains and bind specific dyes like Congo red and Thioflavin-T. Clinical amyloid imaging with PET-tracer [^{11}C]PIB, which was pioneered by Uppsala University and Uppsala Imanet together with scientists in Pittsburgh, allows senile plaques of living AD brain to be visualized and quantified. Unfortunately the signal early on reaches its maximum and does not further increase as the disease progresses, i.e. there is a marked ceiling effect. More recently soluble A β has been found to correlate better with disease severity, and oligomeric A β assemblies of various molecular sizes shown to elicit toxic effects *in vitro* and *in vivo*.

The aims of the project are to improve clinical PET-imaging, primarily by imaging soluble A β , to allow for assessing disease progression and therapeutic efficacy.

During 2012 we have performed protein engineering of antibody-based ligands for PET-imaging of soluble A β aggregates. Thus, both the intact antibody as well as a F(ab') $_2$ -fragment thereof were studied. The specificity and affinity of the ^{125}I -labelled antibody-based tracers were evaluated using *in vitro* and *in vivo* binding to synthetic soluble and insoluble A β aggregates. Furthermore, we have assessed uptake of the antibody-based tracer in transgenic tg-ArcSwe and tg-Swe mice with and without amyloid plaques, and determined its pharmacokinetic properties in *ex vivo* studies. The uptake of the ^{124}I -labelled antibody based PET-tracers was studied in tg-ArcSwe mice using animal PET/CT. The PET-imaging was performed with transgenic mice of different ages and stage of pathology. To increase the passage over the blood-brain barrier continued protein engineering has been performed. One possibility to increase brain penetration is to cationize the h158-F(ab') $_2$ fragment, which increases its positive surface charge and therefore increase transcytosis over the BBB. A second method is by to enhance the BBB passage by coupling the antibody or the fragment to a transferrin receptor antibody or transferrin recognizing peptide, which results in an active transport of the antibody/fragment into the brain. Both methods have been used and are currently analyzed.

The project is carried out in collaboration with BioArctic Neuroscience AB, with expertise on immunotherapy and antibody engineering, and with scientists at the Department of Public Health and Caring Sciences, Uppsala University, having expertise in animal studies of AD using transgenic animals.

Members of the group during 2012

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Gunnar Antoni, Associate Professor
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Funding

The project is funded by a three year grant from Vinnova (2009 – 2012) with similar funding from GE Healthcare and BioArctic Neuroscience AB. The project is also funded by grants from Alzheimerfonden and Hjärnfonden.

Synthesis and preclinical evaluation of ^{11}C and ^{18}F -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 β -deposits as well as prefibrillar A β assemblies and neurofibrillary tangles and exhibit distinct different emission spectra depending on which protein the molecule is bound to. In this project the prime objective is to label a library of thiophene derivatives with ^{11}C and ^{18}F and to investigate the specificity of binding and the potential of this class of compounds as PET tracers for the study of the different protein deposits found in AD patients. A potential novelty would be to distinguish by diagnostic imaging with PET between amyloid deposits and neurofibrillary tangles. Another interesting opportunity is to study systemic amyloidosis and be able to visualize and quantify amyloid deposits in organs such as, heart, liver, lung and kidney.

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

Two rat whole-body PET studies were performed with the two promising ligands [^{18}F]TPHE and [^{11}C]TPHF on normal rats of normal age, considered to have no amyloid in the brains. Consequently, very little uptake was found in the brains of these rats. Moreover, PET / CT was performed in a healthy female Cynomolgus monkey, assumed to have no amyloid depositions in the brain or elsewhere, to study the distribution of the three ligands [^{11}C]TPHB, [^{11}C]TPHD and [^{18}F]TPHE. These in vivo PET studies were performed to see the general distribution of the ligands and to get sufficient pharmacokinetic data before studying animals with amyloidosis, either in brain or systemic.

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

Members of the group during 2012

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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).

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 2012

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Funding

Swedish Cancer Society (Cancerfonden)

800 000 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 ¹¹¹In-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 2012

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Funding

Swedish Research Council (Vetenskapsrådet) 900 000 SEK

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***In vitro* visualization of carcinoembryonic antigen using bispecific antibodies**

Research Group Leader: Håkan Hall

The carcinoembryonic antigens (CEAs) are glycoproteins that are involved in cell adhesion, and are produced during fetal development. CEA is expressed in some carcinomas, and especially colorectal cancer, and can therefore be detected both in tissue and in blood in patients with this cancer type. CEA is therefore used as a serum marker for the prognosis of colorectal cancer. The present study was undertaken to explore the possibility to use CEA as a marker for colorectal cancer in biopsies using *in vitro* autoradiography with antibodies directed against CEA.

However, the long biological half-life (days – weeks) of most antibodies has hampered the use of radiolabeled antibodies in PET. To overcome this problem, methods of pre-targeting can be used, i.e. to pre-target the tissue with an antibody followed by visualization of the

bound antibody in a second step some days later. We use a trivalent, bispecific binding protein (TF2) consisting of two identical Fab fragments reacting against CEA, linked specifically to a different Fab fragment capable of reacting with a divalent hapten peptide.

In this *in vitro* pre-targeting visualization study we have evaluated the binding of a trivalent bispecific protein TF2 and two radiolabeled (Ga-68 and Ga-67) haptens to cryosections from patients who have undergone surgery for colorectal cancer. *In vitro*, the sections were incubated with TF2 followed one hour later by the subsequent incubation of the radiolabeled hapten peptide. This technique provided imaging of CEA with very high contrast.

Members of the group during 2012

Håkan Hall, Adjunct Professor

Irina Velikyan, Associate Professor

Radiolabelling technology

Development of methods for labelling with synthesis with ^{11}C

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}C and evaluate its usefulness. The importance is based on the technical simplicity compared with the existing methods for labelling synthesis with ^{11}C .

Members of the group during 2012

Gunnar Antoni, Associate Professor

Mats Larhed, Professor

Patrik Nordeman, PhD student

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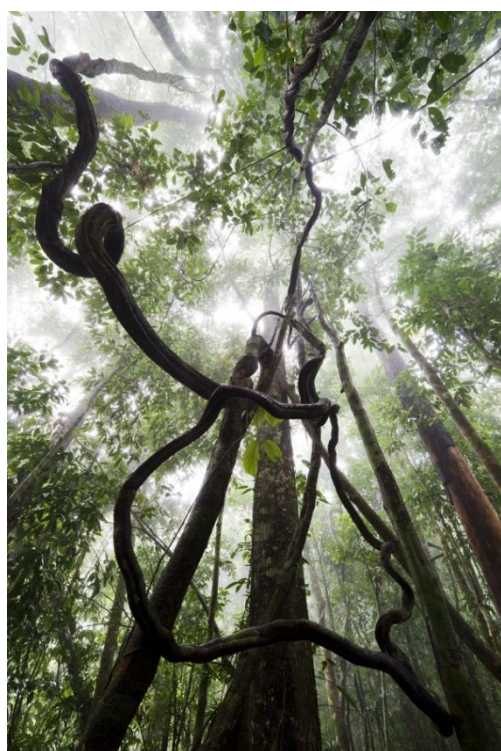
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Pharmacognosy

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

Our research represents a modernization and renewal of a venerable 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 pharmacognosy, 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

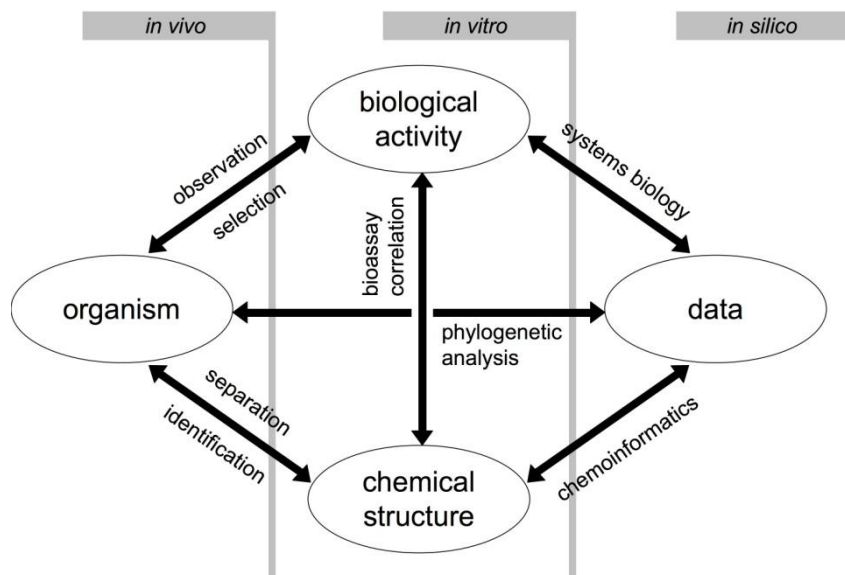


Figure 2. The interdisciplinary nature of pharmacognosy is demonstrated by the explanatory model above (Figure by S. Larsson)

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.

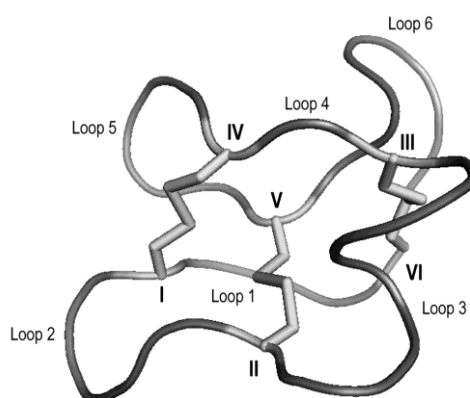


Figure 3. The cyclotide backbone. Note the circular backbone and the cystine knot that define the cyclic cystine knot (CCK) motif. The variable loop regions (marked 1-6) between the cysteines (marked I-VI) are targets for protein engineering in this project. The CCK motif is able to harness a number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

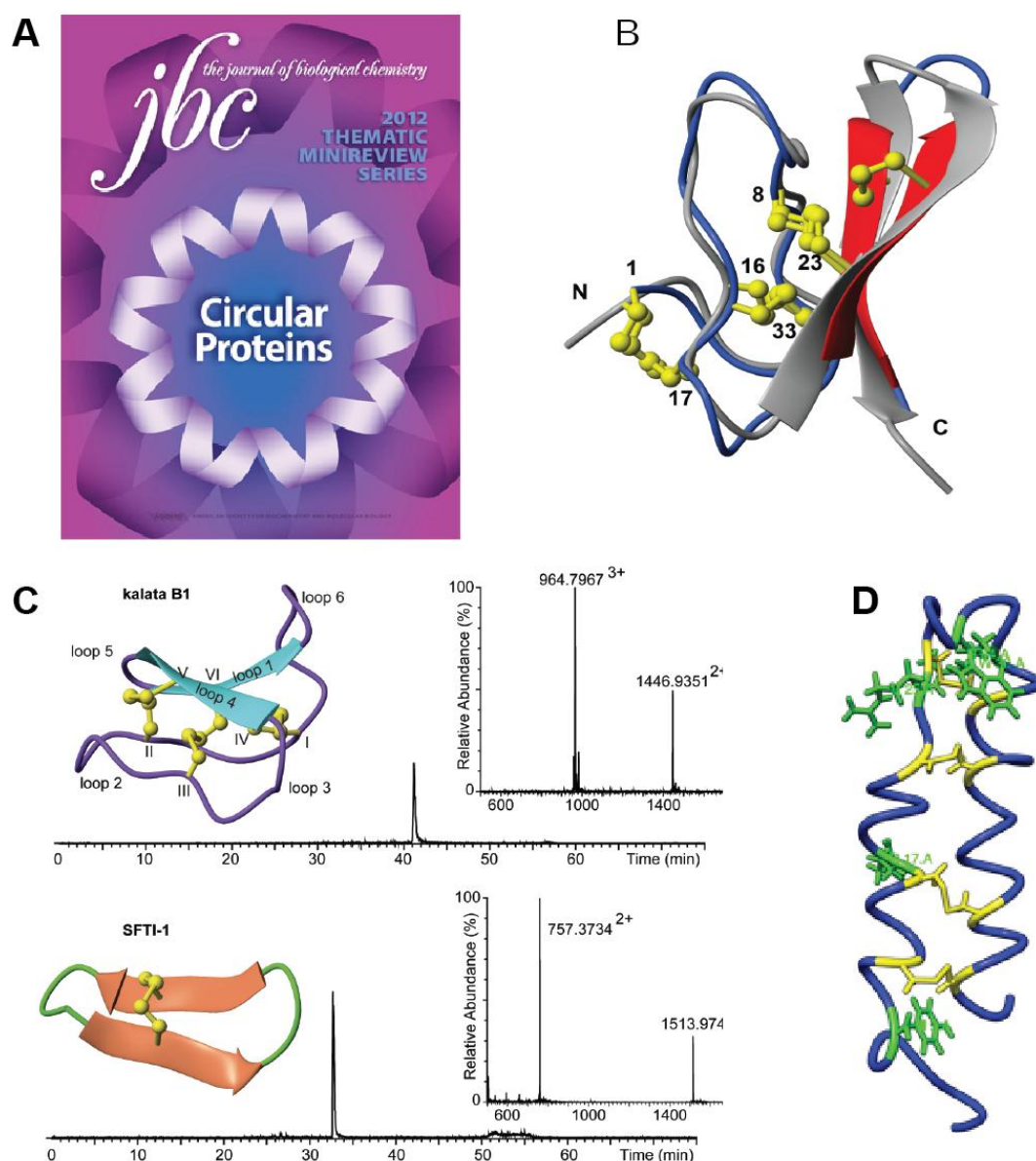


Figure 4. Some research highlights 2012. A) The group contributed with one article in the thematic minireview series on circular proteins published by J Biol Chem (Göransson et al, JBC 2012) B) The antimicrobial peptide isolated from the cactus *Echinopsis pachanoi* has almost an identical structural 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 Ślęzak 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 successfully 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 Göransson group during 2012

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 Ślęzak, 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)

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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 2012

1. Swedish Foundation For Strategic Research, Programme for Future Research Leaders, 1700 000 SEK
2. Swedish Research Council, NT, 850 000 SEK
3. Carl Tryggers Foundation, 240 000 SEK (Post doctoral Fellowship awarded to S. Gunasekera)

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 brucei* 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 trypanosomatids 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 Bayesian 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 eukaryotic homologue, for example isovaleryl-CoA dehydrogenase in the trypanosomads.

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 2012

Cecilia Alsmark, Assistant professor

Anders Backlund, Professor

Anna Koptina, PhD., Post doc

Elisabet Vikeved, MSc, PhD student

Åke Strese, MSc, PhD student

Publications 2010-2012

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

Reviews

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.

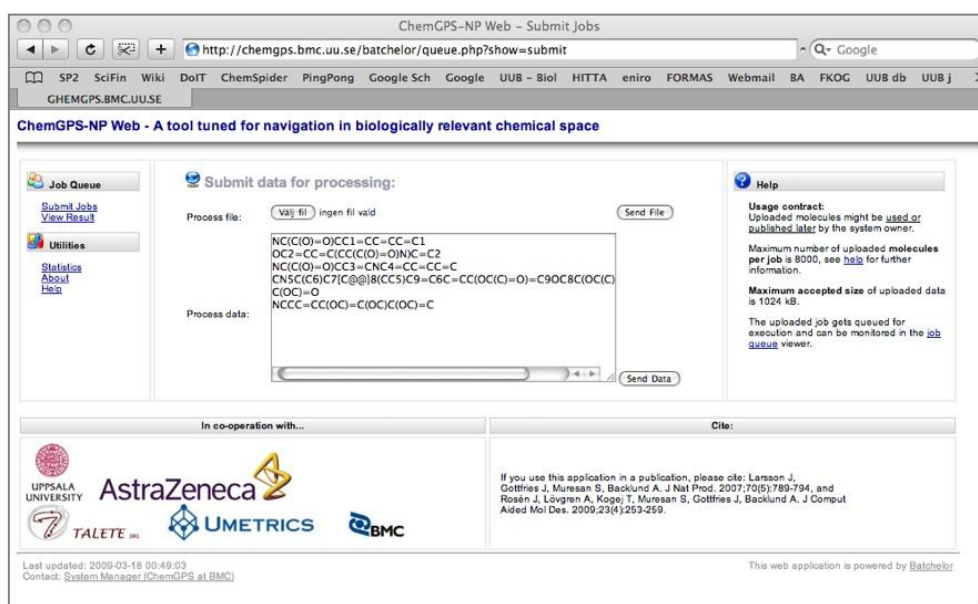


Figure 5. Web interface for ChemGPS-NPweb.

Since the launch in May 2008 more than 6 million compounds originating from more than 4000 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 derivatization 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 derivatising could be suggested.

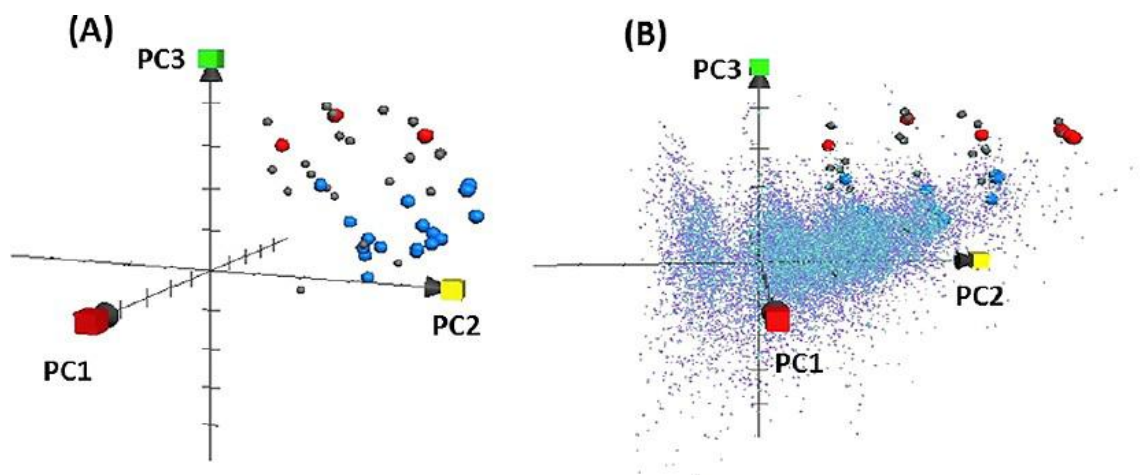


Figure 6. In 6A we can see a set of cytotoxic compounds plotted in chemical property space. Those coloured in red indicates highly potent compounds, those in blue less potent, and the gray compounds with low or intermediate effect. In figure 6B, the tested compounds are related to the ZINC-NP reference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

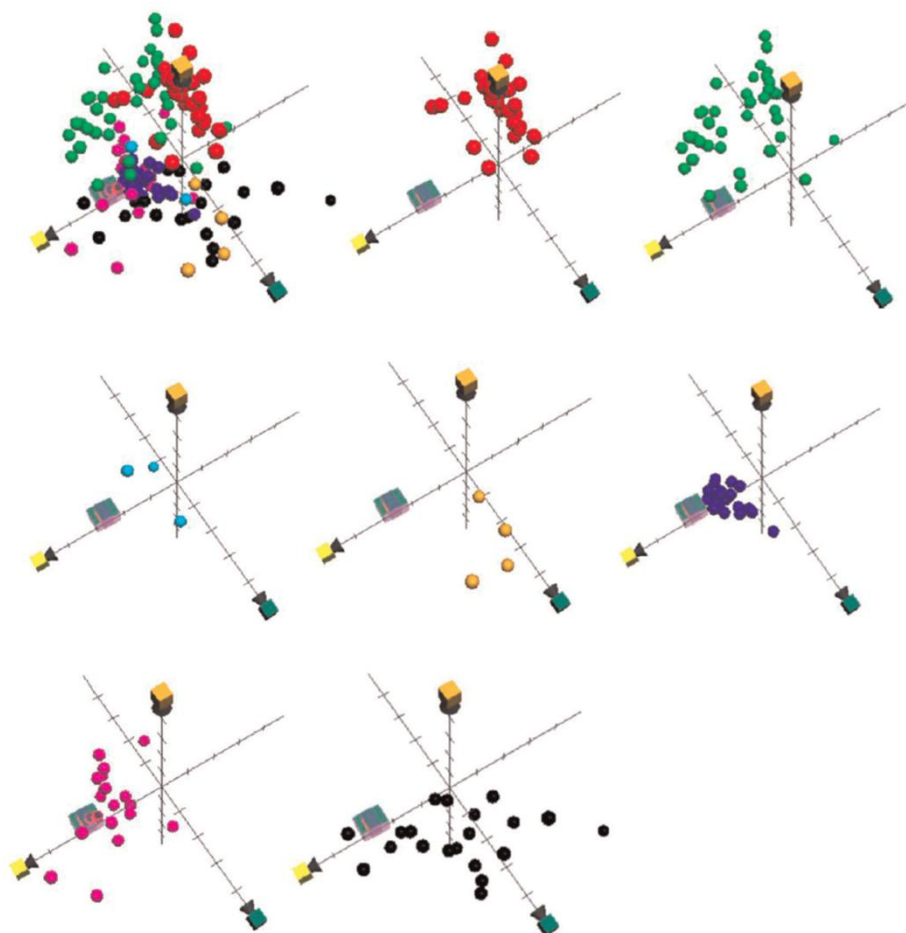


Figure 7. ChemGPS-NP analysis of calanquinone A and denbinobin. Score plot of the three dimensions (principal components 2–4) consisting of PC2 (yellow; aromaticity etc.), PC3 (green; lipophilicity etc.) and PC4 (orange; flexibility/rigidity), from analysis of most potent compounds 6a and 6b as medium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

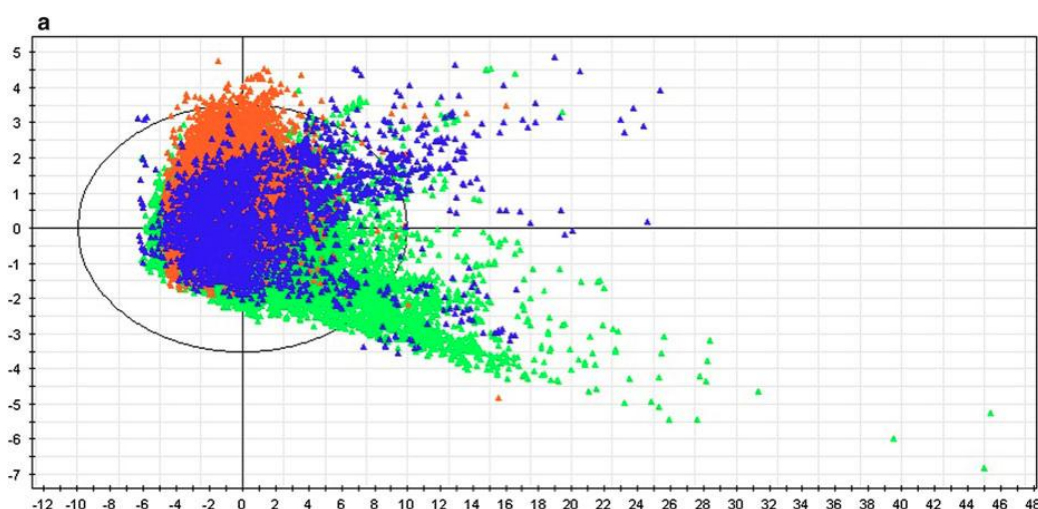


Figure 8. Results from chemographic mapping of marine (blue), terrestrial (green) and druglike (orange) compounds, compiled from literature. Plots clearly demonstrates differential coverage of multi-dimensional chemical property space. From Muigg *et al.*, 2012 in *Phytochemistry Reviews*.

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, was 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 on next page.

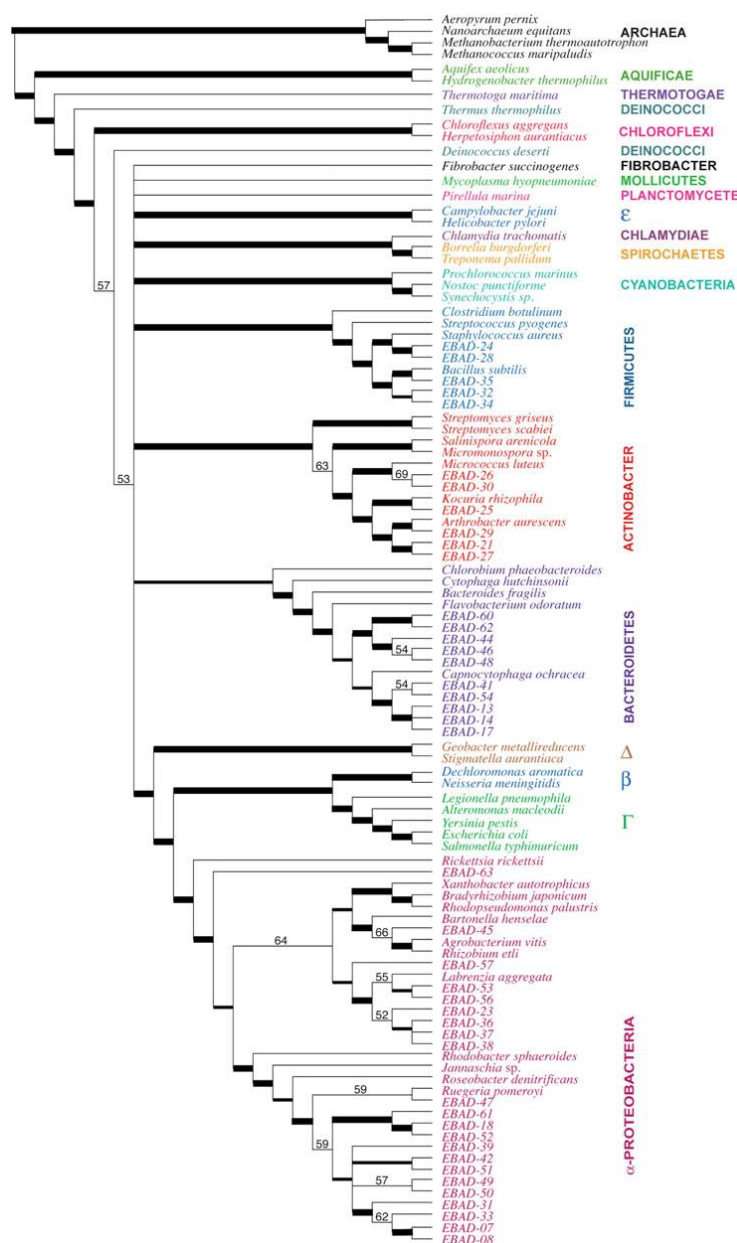


Figure 9. Phylogenetic tree a selected set of reference bacteria, and phylogenetic position of environmental samples from the interior of an *Acyonium* soft-coral (labels EBAD-#). This indicates not only that there is a wide diversity of bacteria living inside the coral, but also that these can be firmly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

Members of the group during 2012

Anders Backlund, Professor

Cecilia Alsmark, Assistant Professor

Christina Wedén, Postgraduate researcher, PhD - thesis defended 2004

Sonny Larsson, Postgraduate researcher, PhD - thesis defended spring 2007

Anna Koptina, Postgraduate researcher, PhD

Elisabet Vikeved, MSc, PhD student

Åke Strese, MSc, PhD student

Publications 2010-2012

1. Wedén, C., Bai, G., Göransson, U., Perez-Aronsson, A., Burman, R. Borg-Karlsson, A.-K., and Backlund, A.: The Swedish populations of *Tuber aestivum*. in: ATTI 3^o Congresso Internazionale di Spoleto sul Tartufo, ed. D. Domizia. Comunità Montana dei Monti Martani, Serano e Subasio, Spoleto, Italy. ISBN 9788-890512209, p. 213-217 – 2011.
2. Alsmark, C., Strese, Å., Wedén, C., and Backlund, A.: Microbial diversity of *Alcyonium digitatum*. *Phytochemistry Reviews* DOI 10.1007/s11101-012-9229-5 – 2012
3. 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.
4. Muigg, P., Rosén, J., Bohlin, L. and Backlund, A.: In silico comparison of marine, terrestrial and synthetic compounds using ChemGPS-NP for navigating chemical space. *Phytochemistry Reviews* DOI 10.1007/s11101-012-9256-2 – 2012
5. Frédérick, R., Bruyère, C., Vancraeynest, C., Reniers, J., Meinguet, C., Backlund, A., Masereel, B., Kiss, R., and Wouters, J.: Novel trisubstituted harmine derivatives with original in vitro anticancer activity. *Journal of Medicinal Chemistry* 55, pp 6489-6501 DOI 10.1021/jm300542e – 2012.

6.

Reviews 2010-2012

1. L. Bohlin, U. Göransson, C. Alsmark, C. Wedén, A. Backlund: Natural products in modern life science. *Phytochemistry Reviews* 9; 2010, 279-301. DOI 10.1007/s11101-009-9160-6.

Books and general/popular science 2010-2012

1. A. Backlund: Topical chemical space in relation to biological space. in: Comprehensive Natural Products II Chemistry and Biology, eds. L. Mander & H.-W. Lui. Elsevier, Oxford vol. 3; 2010, 47-79.
2. A. Backlund, A. Lövgren, R. Sedzik, and J. Rosén: SIMCA-QP – a part of ChemGPS-NPweb. in: UMETRICS application note 22
<http://www.umetrics.com/Content/Document%20Library/AppNotes22SIMCA-QP.ept> – 2010.
3. L. Bohlin, C. Alsmark, U. Göransson, M. Klum, C. Wedén and A. Backlund: 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. 2011.

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-HT₄ 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 2011

Lars Bohlin, Professor

Ulf Göransson, Associate professor
Stefan Svahn, PhD student

Publications 2010-2012

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.

Reviews 2010-2012

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

Agencies that support the work/funding

VINNOVA 250 000 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 cyclooxygenase-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 2012

Lars Bohlin, Professor

Anders Backlund, Professor

Ulf Göransson, Associate professor

Publications 2010-2012

1. Burman, R. Svedlund, E. Felth, J. Hassan, S. Herrmann, A. Clark, R.J. Craik, D.J., Bohlin, L. Claeson, P. Göransson, U. Gullbo, J.: Evaluation of toxicity and anti-tumour activity of cycloviolacin O2 in mice. *Biopolymers* 94; 2010, 626-634.
2. Gerlach, S.L., Burman, R. Bohlin, L. Mondal, D. Göransson, U.: Isolation, Characterization, and Bioactivity of Novel Cyclotides from the Micronesian Plant, *Psychotria leptothyrsa* var. *longicarpa*. *Journal of Natural Products*. 73(7); 2010, 1207-13.
3. 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.
4. Roggen H, Charnock C, Burman R, Felth J, Larsson R, Bohlin L, et al. Antimicrobial and antineoplastic activities of Agelasine analogs modified in the purine 2-position. *Archiv der Pharmazie*. 2011; 344(1):50-55.
5. 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-.
6. Hassan S, Laryea D, Mahteme H, Felth J, Fryknäs M, Fayad W, et al. Novel activity of acriflavine against colorectal cancer tumor cells. *Cancer Science*. 2011; 102(12):2206-13.

Reviews 2010-2012

1. H.R. El-Seedi, M.A. El-Barbary, D.M.H. El-Ghorab, L. Bohlin, A.K Borg-Karlsson, U. Göransson, R. Verpoorte: Recent insights into the biosynthesis and biological activities of natural xanthenes. *Current Medicinal Chemistry*, 17; 2010, 854-901.
2. Bohlin L, Göransson U, Alsmark C, Wedén C, Backlund A. Natural products in modern life science. *Phytochemistry Reviews*. 2010; 9(2):279-301.
2. L. Bohlin, U. Göransson, C. Alsmark, C. Wedén, A. Backlund: Natural products in modern life science. *Phytochemistry Reviews*. 9(2); 2010, 279-301.

Undergraduate Teaching

The Department of Medicinal Chemistry is involved in teaching at six educational programmes: the Bachelor of Science in Pharmacy programme (180 hp), the Master of Science in Pharmacy programme (300 hp), the Biomedical programme (240 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.

Biomedical programme, 240 hp (Biomedicinarprogrammet)

The Department's contribution to this programme aims at providing fundamental knowledge of drug oriented chemistry and the courses given are General Chemistry (7.5hp) and Organic

and Medicinal Chemistry (15 hp). 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 7th semester is mandatory for about 40 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 2012

Anders Backlund was awarded a position as Honorary Visiting Professor at Kaohsiung Medical University, Taiwan

The Rudbeck medal 2012 from Uppsala University awarded Professor Anders Hallberg

Benzelius Award 2012 (Royal Society of Sciences in Uppsala) awarded Rebecca Fransson

The Study Council at the Pharmaceutical Student Union awarded University lecturer Anja Sandström the 2012 Student Influence Award

STINT (The Swedish Foundation for International Cooperation in Research and Higher Education) awarded 2012 University lecturer Anja Sandström fellowship within the “Excellence in Teaching” program, *to be* placed at Amherst College during fall 2013

List of Staff

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www.ilkk.uu.se

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Division of Pharmacognosy, www.fkog.uu.se

Preclinical PET Platform, pet.medchem.uu.se

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Ahmad Amini

Torbjörn Arvidsson

Victoria Barclay

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Albert Elmsjö

Mikael Engskog

Olle Gyllenhaal

Jakob Haglöf

Mikael Hedeland

Ylva Hedeland

Alexander Hellqvist

Monika Johansson

Anders Karlsson

Curt Pettersson

Axel Rydevik

Matilda Lampinen Salomonsson

Alfred Svan

Eva Strömberg

Annica Tevell Åberg

Niklas Tyrefors

Douglas Westerlund

Organic Pharmaceutical Chemistry

Hiba Aloqheli

Mathias Alterman

Per Arvidsson

Linda Axelsson

Anna-Karin Belfrage

Anne-Marie Benson

Gunilla Eriksson

Ashkan Fardost

Rebecca Fransson

Johan Gising

Anders Hallberg
Anders Karlén
Anna Lampa
Mats Larhed
Gunnar Lindeberg
Martin Lindh
Torbjörn Lundstedt
Linda Åkerbladh
Patrik Nordeman
Anneli Nordqvist
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Francesco Russo
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