university of groningen
faculty of science and engineering
groningen research institute of pharmacy

Analytical Biochemistry & Interfaculty MS Center

Annual Report 2018

Prof. Dr. Rainer Bischoff
Prof. Dr. Peter Horvatovich
Dr. Hjalmar Permentier

January 16, 2019

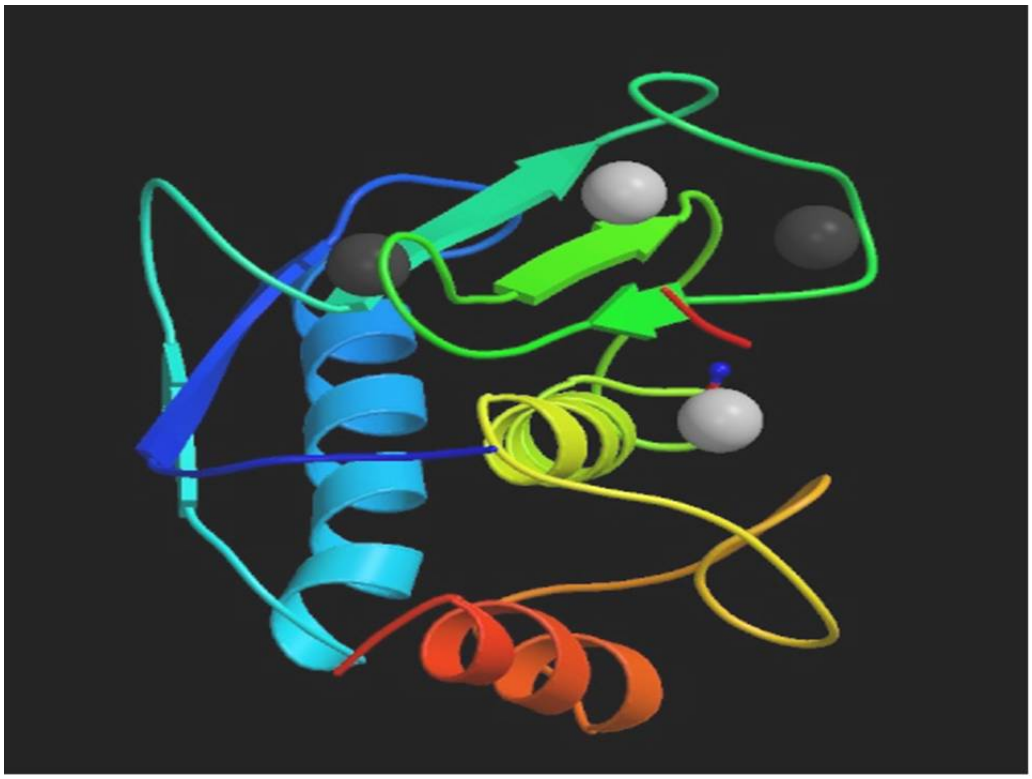


Table of Contents

From left to right and from back to front:
Wenxuan Zhang, Ali Alipour, Margot Jeronimus-Stratingh, Marcel de Vries, Jos Hermans, Peter Bults, Jolanda Meindertsma, Hjalmar Permentier, Janine Stam, Rainer Bischoff, Andrea Kurtinović, Jan Willem Meints, Saskia Sokoliova, Peter Horvatovich, Adelina Dinter, Yang Zhang, Alienke van Pijkeren, Rien Leuvenink, Sara Russo, Oladapo Olaleye, Susan Visscher, Xiaobo Tian, Ydwine van der Veen, Baubek Spanov, Frank Klont, Victor Bernal, Jiaying Han, Natalia Govorukhina
Not on photo:
Karin Wolters, Andrei Barcaru, Marcel Kwiatkowski, Dirk-Jan Reijngoud, Nico van de Merbel, Thomas Cremers, Alex Sanchez, Walid Maho, Julia Aresti Sanz, Rik Beernink

Analytical Biochemistry Group
Prof. Dr. Rainer Bischoff
Phone: (+31)-50-363-3336
E-mail: R.P.H.Bischoff@rug.nl
Prof. Dr. Peter Horvatovich
E-mail. P.L.Horvatovich@rug.nl
Website: www.biomac.nl

Interfaculty MS Center
Dr. Hjalmar Permentier
Phone: (+31)-50-363-3262
E-mail: H.P.Permentier@rug.nl
Website: http://mscenter.webhosting.rug.nl/tiki-index.php

Members of the Research Groups

Staff

Prof. Dr. Rainer Bischoff
Prof. Dr. Peter Horvatovich
Prof. Dr. Nico van de Merbel (PRAHS)
Prof.Dr. Thomas Cremers (per June 1, 2018, CAN Holding)
Dr. Natalia Govorukhina
Jos Hermans
Jan Willem Meints
Jolanda Meindertsma (secretary; 0.4 fte)

Interfaculty Mass Spectrometry Centre

Dr. Hjalmar Permentier
Annie van Dam (0.5 fte, retired per June 1, 2018)
Margot Jeronimus-Stratingh (0.5 fte, retired per Jan. 1, 2019)
Marcel de Vries (UMCG)
Walid Maho (0,8 fte, per September 1, 2018)

Post-doctoral researchers

Dr. Karin Wolters (UMCG)
Dr. Marcel Kwiatkowski (GRIP, Pharmacokinetics, Toxicology & Targeting)
Dr. Andrei Barcaru (UMCG)

Ph.D. students

Jorge Andres Gil Quintero (thesis defense September 14, 2018)
Frank Klont (thesis defense scheduled for Feb. 08, 2019)
Jiaying Han
Victor Bernal Arzola
Peter Bults (PRAHS)
Wenxuan Zhang (UMCG)
Yang Zhang
Xiaobo Tian
Ali Alipour
Alienke van Pijkeren (UMCG)
Wadha Abushareeda (per April 1, 2018) (Qatar Antidoping Lab)
Julia Aresti Sanz (per April 16, 2018) (shared PhD with Microbial Physiology, GBB/RUG)
Baubek Spanov (per May 1, 2018)
Oladapo Olaleye (per June 1, 2018)
Alex Sanchez Brotons (per June 1, 2018)
Saskia Sokoliova (per July 1, 2018) (shared PhD with the Stratingh Institute of Chemistry/RUG)
Sara Russo (per September 1, 2018)
Janine Stam (per October 1, 2018)
Rik Beernink (IQ Products)

Research Students

Robin Soemopawiro (until May 24, 2018)
Naomi Sanders (until January 1, 2018)
Marrit Hadderingh (until February 1, 2018)
Hedwich Meindertsma (until February 1, 2018)
Tim Lijster (until September 13, 2018)
Susan Visscher (per September 1, 2018)
Rien Leuvenink (per November 1, 2018)
Andrea Kurtinović (per November 26, 2018)

Adelina Dinter (per December 03, 2018)

Guests

Dr. M. Rebecca Heiner-Fokkema (UMCG)
Theo Boer (UMCG)
Prof. Dr. Dirk-Jan Reijngoud (UMCG)
Ydwine van der Veen (UMCG)
Jonatan Erikson, PhD student from University of Lund (October-November 2018)
Prof. Dr. Julia Pavan Solver, University of Sao Paulo (October-December 2018)

Overview 2018

2018 was a year of change, especially for the Interfaculty Mass Spectrometry Center (IMSC). Two experienced technicians, Annie van Dam and Margot Jeronimus-Stratingh retired after working for the IMSC for many years. This meant that a lot of experience had to replaced, which is naturally not possible from one day to the other. Fortunately, we found a candidate to take over as technician of the IMSC, Walid Maho. While not as experienced as Annie and Margot, Walid started with a lot of enthusiasm and has already learned many 'tricks of the trade' over the last months. We are confident that, with the help of the IMSC team under supervision of Hjalmar Permentier and others in the group, things will run smoothly in 2019.

There was also considerable change in the Department of Analytical Biochemistry (AB), as a new generation of PhD students started. This necessitated a considerable effort from staff members and postdocs to get everybody started in a new environment. In addition, 2018 saw a number of bachelor and master's students doing projects in the group as well as visiting scientists. We would like to thank everybody, who made special efforts to assure that everyone was welcomed in the group and helped to move ahead with their respective projects.

The year 2018 was also quite successful in terms of output, especially peer-reviewed publications. To name just one example, we established a number of validated LC-MS assays for the soluble Receptor of Advanced Glycation Endproducts (sRAGE) and applied them to samples from clinical studies on Chronic Obstructive Pulmonary Disease (COPD) in close collaboration with Nick ten Hacken (UMCG, GRIAC). This led not only to new insights into circulating sRAGE levels and how their measurement depends on the method and notably on how samples are prepared but also on identifying an important preanalytical factor, acute cigarette smoke exposure, which affects these levels significantly. Recognizing such factors and taking them into account in future biomarker studies will hopefully resolve some of the discrepancies in sRAGE measurements related to COPD and help to qualify sRAGE as a biomarker for lung function decline due to emphysema.

There were a number of other collaborative projects, notably in the area of proteogenomics and bioinformatics (Peter Horvatovich), the combination of electrochemistry and mass spectrometry (Hjalmar Permentier and Rainer Bischoff) and therapeutic proteins (Rainer Bischoff and Nico van de Merbel). While some of them are still in their start-up phase, some have already delivered very interesting results, such as the work on the electrochemical transformation of alkaloids to precursors for the production of value-added pharmaceuticals.

As our research lines rely on sophisticated and expensive infrastructure, it is critical to acquire funding for investments. One such grant was the X-omics (speak 'CrossOmics) program, which is part of the National Roadmap for Large-Scale Infrastructures. Just before Christmas, we received the green light to invest in a new high-resolution mass spectrometer, the necessary periphery (e.g. an LC system) and software. This investment will allow us to continue with the work on therapeutic proteins (EU-ITN project) as well as to strengthen our work on the use of data-independent acquisition (DIA) modes for large-scale biomarker research. Especially the later will require dedicated and highly sophisticated data processing and data analysis approaches, a project that will be driven by Peter Horvatovich.

To close, I would like to mention a number of long-term collaborations that we have had over the years and which have become integral parts of our research. These are the fruitful collaboration with PRA Health Sciences (PRAHS) on therapeutic proteins, with the Erasmus Medical Center in the field of biomarkers, with IBM in the area of computational mass spectrometry, with Lund University on the Cancer Moonshot project and with Twente University on electrochemistry-mass spectrometry.

We hope you'll enjoy reading this Annual Report and wish you a successful year 2019. Please contact us in case you see possibilities for collaborations.

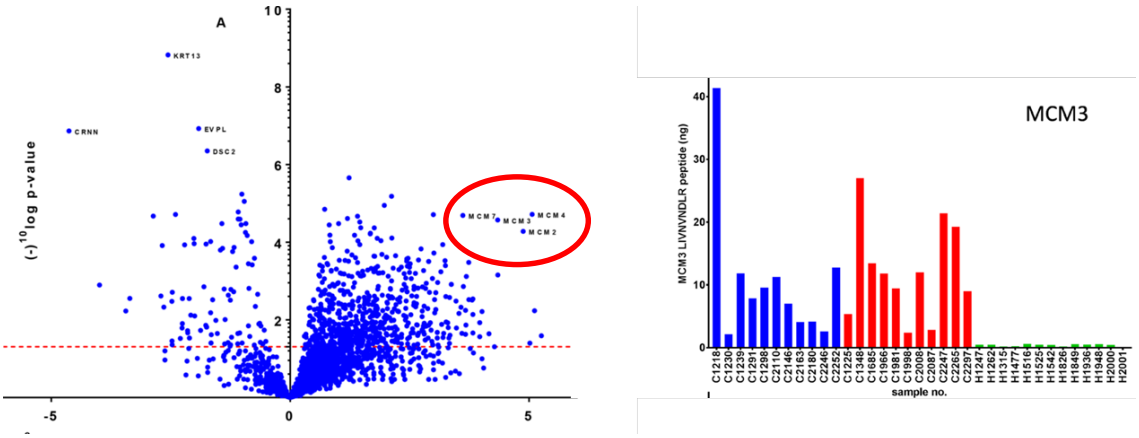
Rainer Bischoff, Peter Horvatovich & Hjalmar Permentier

Research Projects

1. Biomarkers

1.1 Cervical Cancer Biomarkers

The cervical cancer project progressed significantly in 2018, despite limited funding. This is primarily due to the work of our colleagues at the Erasmus MC in Rotterdam (Çoskun Güzel and Theo Luider), who advanced with the analysis of proteins from defined tissue areas obtained by laser microdissection. A comparison of tumor tissue with healthy epithelium and stroma showed a clear upregulation of proteins of the Minichromosome Maintenance Complex (MCM) next to other proteins that must still be investigated. Confirmation of this upregulation was obtained in tissue biopsies and more recently in leftover material from the regular population screen by cytology (so-called Pap smears). This gives us new leads to follow up and new reasons for continuing this project.



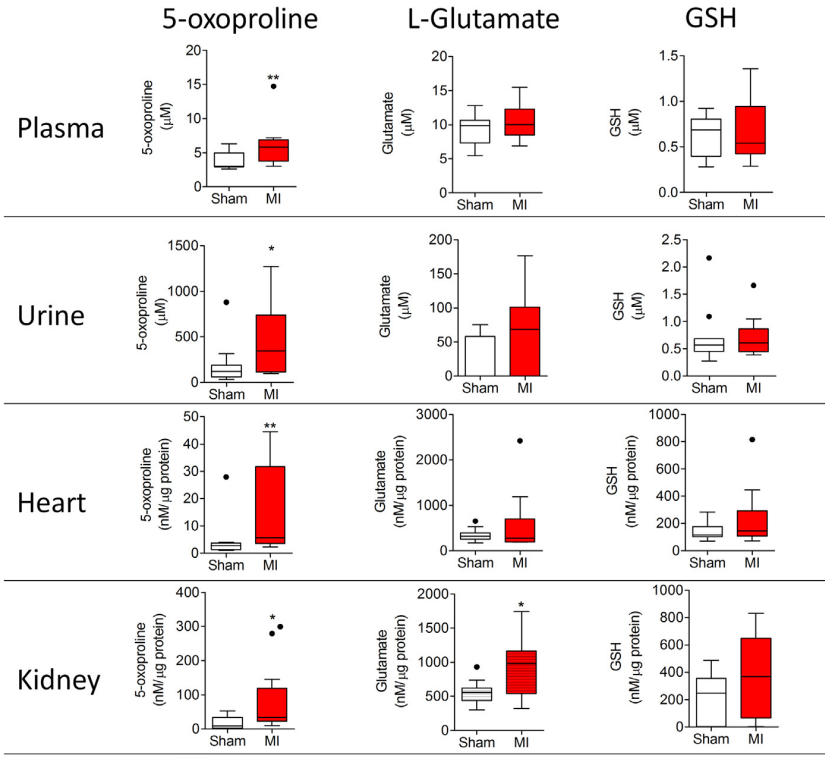
Left: Proteomics analysis of tissue from early-stage cervical cancer versus healthy epithelium showing (amongst others) upregulation of proteins constituting the MiniChromosome Maintenance (MCM) complex. Right: Targeted LC-MS analysis of MCM3, one member of this complex, in tissue biopsies by parallel reaction monitoring based on a signature peptide (blue, early-stage cancer; red: late-stage cancer; green: healthy).[1]

1.2 Heart Failure Biomarkers

After having successfully established in 2017 that the enzyme 5-oxoprolinase (OPLAH) is a critical factor in determining outcome after heart failure, we established an LC-MS method to follow all members of the glutathione cycle in different organs and body fluids to see how heart failure affects this enzyme system. To this end we compared the effect of an induced heart attack in OPLAH knock-out mice with wild-type animals and related it also to heart failure with a preserved ejection fraction. All of the animal experiments were performed by Atze van der Pol in the Department of Cardiology at the UMCG (Peter van der Meer) while the analytical work was done by Andres Gil of the Department of Analytical Biochemistry. It was interesting to see that 5-oxoproline was not only increased in heart tissue and plasma, as established before, but also in urine and kidney tissue, opening further possibilities for evaluating this biomarker, notably in urine.

—

[1] Güzel et al., Oncotarget 2018, 9, 18128-18147.



Levels of 5-oxoproline and two other metabolites of the glutathione cycle in plasma, urine, heart tissue or kidney tissue in mice undergoing a Sham operation or an Induced Myocardial Infarction (MI).[2]

1.3 Building a lipidomics analysis platform

In 2018, we continued the development of a lipidomics analysis platform by implementing an XCMS data pre-processing platform to study the effect of inborn errors of metabolism on lipid profiles in plasma and cultured fibroblasts from children (collaboration with Dirk-Jan Reijngoud & Folkert Kuipers, Department of Paediatrics and Metabolic Disease, UMCG) and Rebecca Heiner-Fokkema & Ido Kema (Department of Laboratory Medicine, UMCG)). Wenxuan Zhang (PhD student), Xiaodong Feng (PhD student) and Andrei Barcaru (postdoctoral researcher) collaborated closely to realize this project. The analytical platform is operational and the XCMS workflow will be operational in 2019. Both are currently being applied to establish lipid profiles from cultured fibroblasts derived from children with known and unknown errors of metabolism.

1.4 COPD Biomarkers

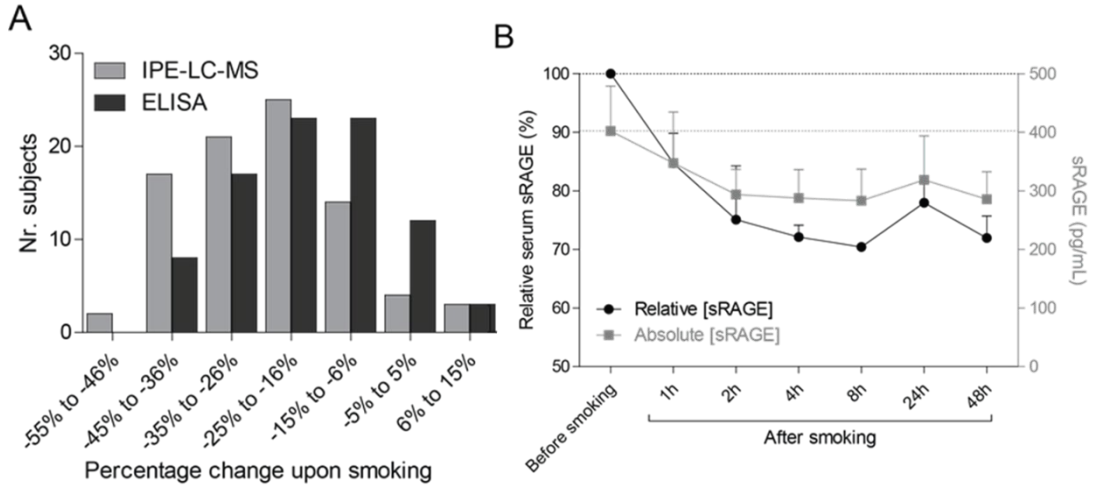
The project on the soluble Receptor of Advanced Glycation Endproducts (sRAGE) advanced considerably in 2018. Three independent analytical methods were developed with enrichment by immunoaffinity, affimer-based affinity and strong cation-exchange solid-phase extraction (SPE). Not surprisingly, results differed from each other and from the commercial ELISA. This is one of the first studies, where a single biomarker is measured with 4 different methods that are based on different sample preparation and/or measurement principles.

In addition, we found a so far unrecognized preanalytical, confounding factor, acute exposure to cigarette smoke, that affects sRAGE levels in serum significantly. This may be one reason why results of sRAGE as predictive biomarker for lung function decline due to emphysema are discrepant. We hope that some of these discrepancies will be resolved by taking this confounding factor into account. The analytical work was performed by Frank Klont (PhD student) in collaboration with Daan Pouwels and Nick

—

[2] Gil et al., Journal of Pharmaceutical and Biomedical Analysis 2018, 160, 289-296.

ten Hacken (UMCG, GRIAC) as well as with Russ Bowler (National Jewish Health Center, Denver, Co, USA).



Smoking three cigarettes immediately decreases serum sRAGE (soluble receptor for advanced glycation end-products) levels for up to 48 hours. (A) The relative change in serum sRAGE levels in patients with chronic obstructive pulmonary disease (n = 13) and control subjects without airway obstruction (n = 75) induced by smoking three cigarettes within 1 hour, measured by immunoprecipitation in 96-well ELISA format–coupled liquid chromatography mass spectrometry (IPE-LC-MS) and the DuoSet ELISA kit from R&D Systems. (B) Absolute and relative serum sRAGE levels in three healthy individuals before and after smoking three cigarettes within 1 hour, measured by ELISA. Blood samples were taken 1, 2, 4, 8, 24, and 48 hours after smoking (n=6, SEM).[3]

2. Computational Mass Spectrometry

The analysis of complex mixtures with hyphenated analytical methods like LC-MS/MS or the imaging of compound distributions in tissue sections with mass spectrometry generates enormous amounts of data corresponding to several tens of thousands of compounds per sample. In order to be able to compare a limited number of samples in typical biomarker discovery projects, it is thus vital to reduce the number of variables without losing relevant information. The way from the raw data to the so-called "clean data" ready for statistical analysis is called data pre-processing. Development of efficient and reliable data pre-processing algorithms is one of the main research lines of Peter Horvatovich, which requires knowledge of signal processing, analytical chemistry, mathematics and statistics to develop and assess the performance of data pre-processing steps as well as an understanding of the structure of the data and the analytical procedures through which artefacts may have been generated. Application of the developed algorithms to clinical translational research, such as biomarker discovery and proteogenomics data integration, is on top of the agenda.

2.1 Threshold Avoiding Proteomics Pipeline (TAPP) and mass spectrometry imaging data pre-processing

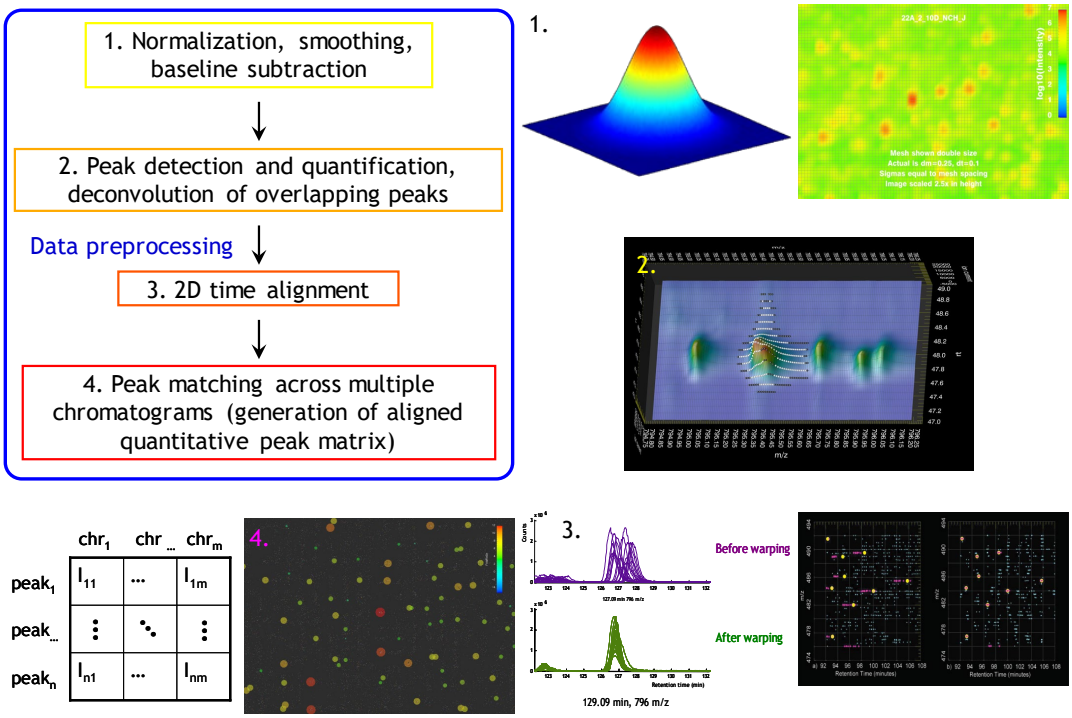
The collaboration with Frank Suits at the IBM Watson Research Center is continuing and was recognized as a "major achievement in science" by IBM. This recognition opens the way to release an open-source version of the Threshold Avoiding Proteomics Pipeline (TAPP), as a major resource for the mass spectrometry community, which is planned for early 2019. We are continuing to develop TAPP by linking the annotation of MS/MS scan information and peptide or metabolite identities to isotopologue peaks in the LC-MS/MS data. Efficient and interactive visualisation of large-scale LC(GC)-MS(/MS) data using

—

[3] Pouwels et al., Am J Respir Crit Care Med 2018, 198, 1456-1458.

advanced GPU programming such as OpenGL has bene prototyped and will be finalised in 2019. Finalisation of this platform and adoption of the pipeline to process data-independent-acquisition (DIA) LC-MS/MS and GC-MS(/MS) data will be the next goal to reach in 2019/2020. The development of TAPP got substantial support by the start of the PhD project of Alexander Sanchez Brotons and the collaboration with Frank Suits (IBM), Karel Gerbrands (voluntary work) and Andrei Barcaru (UMCG) in collaboration with Ido Kema and Stephan Bakker (UMCG).

In collaboration with the University of Lund and Frank Suits, we are further developing an imaging data pre-processing pipeline with the aim to process the complete 4(5)-dimensional mass spectrometry imaging data cube, as acquired with Orbitrap mass analyzers, without any data reduction. The collaboration with Lund has the goal to reveal the distribution of administrated drugs in animal tumour models. The development work is currently performed by Jonatan Eriksson, a PhD student at Lund University. Recently we obtained data from the group of Jeroen Kool and Erika Amstalden (VU, Amsterdam) acquired with a QTOF instrument analyzing protein distributions in animal tissue.



Main steps of the Threshold Avoiding Proteomics Pipeline (TAPP), which performs raw data smoothing and resampling to achieve uniform resolution sampling in the MS dimension (1), isotopologue peak detection and quantification (2), retention time alignment of peaks between chromatograms using the Warp2D algorithm (3) and matching of peaks using retention time and m/z of isotopologue peaks between chromatograms (4).

2.2 Chromosome Centric Human Proteome Project

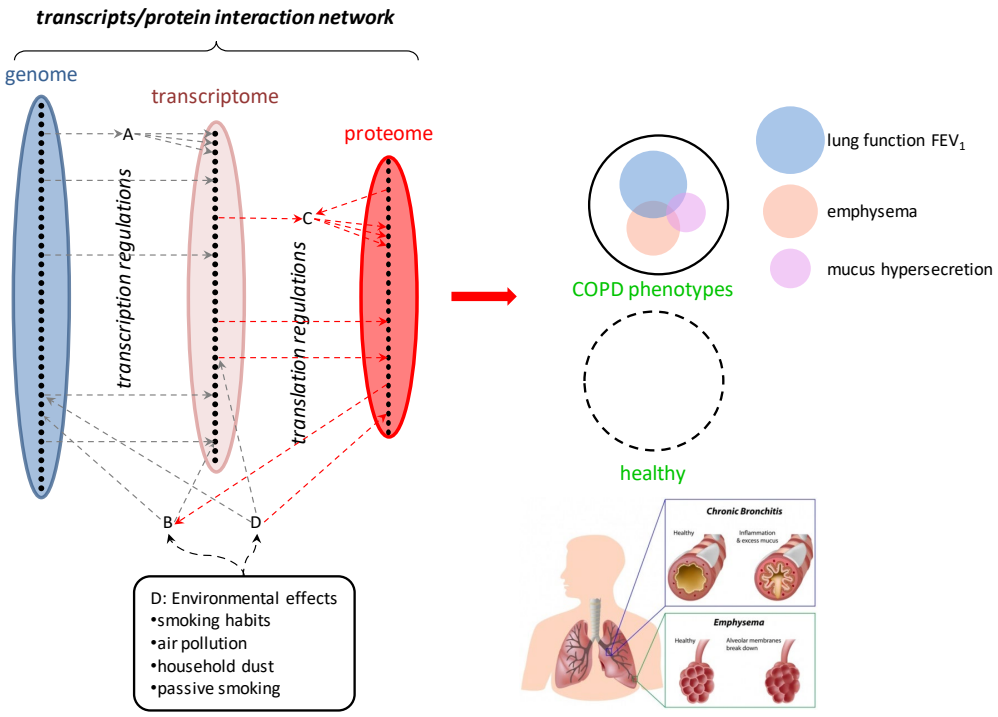
Peter Horvatovich is actively involved in the Chromosome Centric Human Proteome Project (C-HPP) as PI of the Chromosome 5 team and Secretary General of the C-HPP. The C-HPP is an initiative of the Human Proteome Organization (HUPO), which has the goal to catalogue all protein parts of the human proteome. C-HPP events and news are actively reported on the Wiki edited by Peter (http://c-hpp.web.rug.nl). As of October 2017, Peter Horvatovich is also editing C-HPP news for HUPOST, the monthly HUPO newsletter (https://www.hupo.org/HUPOST).

2.3 Proteogenomics data integration for COPD and head and neck cancer

Proteogenomics data analysis integrating mRNA and proteomics data forms another important research line. Data integration is based on constructing patient- and sample-specific protein sequence databases for LC-MS/MS-based peptide/protein identification using mRNA sequence data measured in the same sample. This project was initiated in collaboration with Victor Guryev (ERIBA, UMCG) with participation of colleagues from the Groningen Research Institute on Asthma and COPD (GRIAC; Corry-Anke Brandsma, Maarten van de Berge, Dirkje Postma and Wim Timens) working at the Pulmonology and Pathology Departments of the UMCG. The project has the aim to perform proteogenomics analysis of human lung tissue and human fibroblast cells of COPD patients and controls to identify patient-specific and disease-associated protein forms related to the pathophysiological, molecular mechanisms underlying COPD. A manuscript from this work based on 8 control and 10 COPD stage IV patients is ready for submission and extension of this study to analyse 120 human lung tissues from controls and COPD stages I-IV is planned for 2019/2010.

Another proteogenomics project is ongoing in collaboration with György Halmos and Renee Verhoeven (Head and Neck Department, UMCG) which has the aim to reveal the proteome/transcriptome profile differences between young and elderly head and neck cancer patients. A pilot project was started with the support of Frank Klont to benchmark various protein extraction approaches using three different types of human tissues. In another pilot study, tumor and control tissues were collected and analyzed by next generation sequencing (Illumina) and LC-MS/MS proteomics to compare 10 young and 10 elderly patients with laryngeal squamous cell carcinomas. Proteogenomics integration of the collected data will be a major goal for 2019.

Another project requiring proteogenomics data integration is the EU-funded PROMETOV project which aims to reveal the tumor heterogeneity of ovarian cancer (see section 2.4 for details). We aim to develop this research line further and address other clinical cancer research projects.



Proteogenomics data integration, combining transcriptomics and proteomics profiles of human lung tissue of COPD patients and controls, is key to advance research in revealing the molecular mechanism of COPD onset and development. In a pilot study we analysed transcriptomes and proteomes of human lung tissue from 10 COPD stage IV and 8 control individuals.

2.4 Revealing tumor heterogeneity in ovarian cancer

PROMETOV is an EU-funded TRANSCAN-2 project co-financed by the Dutch Cancer Society (KWF), which has the aim to assess the heterogeneity of primary and metastatic ovarian tumors. This project involves collaborations with several European partners from Germany, Turkey, the UK, Slovenia, Israel and Estonia and one local partner (Kathrin Thedieck, Department of Pediatrics, UMCG). In this project we develop a robust quantitative phosphoproteomics pipeline, which enables us to assess protein phosphorylation changes in tumor tissue. Besides, we will generate high quality proteomics data of primary and metastatic ovarian cancer tissues using a TMT-based stable-isotope chemical labelling approach. Another task of our group is to participate in the integration of multi-omics (phosphopeptide, protein, transcriptomics, tryptophan metabolite) data. The PhD student Yang Zhang, who started in January 2017, works on this project and is co-supervised by Kathrin Thedieck, Natalia Govorukhina, Marcel Kwiatkowski and Alexander Heberle (Department of Pediatrics, UMCG).

2.5 Assessment of mycotoxin exposure of the Qatari population and use of full MS scan data in antidoping analysis

The goal of this project is to identify molecular markers in human blood for foodborne mycotoxin intoxication and to assess the risk of mycotoxin exposure of the Qatari population. This project is performed in collaboration with researchers (Aishah Latif, Thomas Michael Harvey, Morana Jaganjac) of the Anti-Doping Laboratory of Qatar (ADLQ) funded by the Qatar National Research Fund (QNRF). The effect of mycotoxin intoxication is first studied in rats to identify mycotoxin exposure markers in blood. These markers will be subsequently used to assess mycotoxin exposure in humans. Our laboratory has the role to pre-process data of the untargeted LC-MS/MS mycotoxin analyses and to perform the statistical risk assessment based on the obtained data. Andrei Barcaru (UMCG), postdoctoral scientist with chemometric, statistics and programming expertise is supporting this project. Another collaboration with ADLQ and Costas Georgakopoulos involves improvement of the detection of steroids and other doping agents using hyphenated high-resolution GC-MS and LC-MS profiling approaches.

2.6 Identification of metabolic changes in patients with inborn metabolic errors

Inborn errors of metabolism are genetic mutations perturbing food and energy metabolism, which have a detrimental effect on patient health. The goal of this project is to develop a data processing pipeline for organic acid GC-MS data and to develop a statistical method, to identify changes in the metabolite profiles of patients compared to profiles of clinically matched controls. This work is performed in collaboration with Rebecca Heiner-Fokkema (Clinical Chemistry, UMCG). Andrei Barcaru, a postdoctoral scientist from the UMCG, is main collaborator in this project.

2.7 Cancer Moonshot Project for personalised diagnosis and treatment of melanoma patients.

The Cancer Moonshot Project has the goal to integrate proteomics data into clinical cancer research to provide a breakthrough in cancer diagnostics and treatment. György Marko-Varga at the Centre of Excellence in Biological and Medical Mass Spectrometry (CEBMMS) at Lund University (Sweden) is leading a Cancer Moonshot Project focussing on melanoma. Peter Horvatovich has an honorary scientist position at Lund University to supervise the data pre-processing and data analysis parts. The Cancer Moonshot Project at CEBMMS has the aim to profile more than 4 000 samples from melanoma patients over the next 5 years. The role of our group is to support the high-throughput

data analysis of LC-MS/MS proteomics data, proteogenomics data integration, statistical analyses of the collected molecular profiles and clinical metadata and to participate in the supervision of a PhD student (Jonatan Erikson).

2.8 Network analysis to support the understanding of molecular mechanisms in biological systems

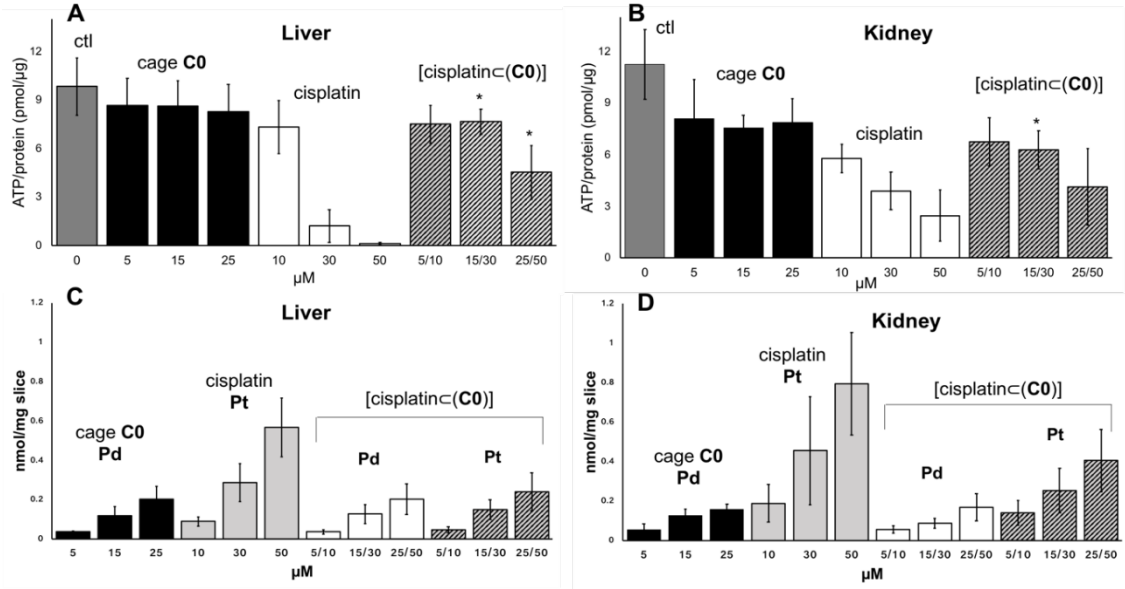
Victor Bernal started his PhD in July 2016 on a project awarded by the Data Science and System Complexity theme of the Faculty of Science and Engineering with partial support from Erik Frijlink (GRIP). This project has the goal to develop Bayesian and Relevance (correlation and partial correlation) Network and Machine Learning approaches to identify molecular subnetworks that are learned directly from the molecular profiles and clinical meta-parameters. This project is a collaboration between multiple research groups comprising genomics (Victor Guryev), statistics (Marco Grzegorzyk), pulmonology (GRIAC) and the metabolic signalling laboratory (Kathrin Thedieck). This work resulted in a manuscript on correcting partial correlation FDR p-value calculation bias and another manuscript on Relevance or Gaussian Graphical Models for analyzing expression array data of cells from nasal and bronchial epithelial brushes. Plans in 2019 are to develop multi-omics data integration through Relevance Network modelling using mRNA and proteomics data and to develop non-equidistant Dynamic Bayesian models for mTOR phosphorylation data.

3. Drug Targeting, RNA-based Therapy and Chemoproteomics

3.1 Drug targeting and photocleavable mass tags for protein distribution imaging

The research line on Imaging Mass Spectrometry (IMS) and drug targeting with bioconjugated palladium-based metallacages encapsulating cisplatin, is being pursued by Jiaying Han (PhD student). This project has the goal to develop a novel, sensitive, targeted IMS approach using photocleavable mass tags, which are coupled to a targeting moiety (antibodies or specific peptides) and to develop a bioconjugation strategy for metallacages encapsulating anticancer agents in collaboration with Angela Casini (Cardiff University, UK) and Hjalmar Permentier (Interfaculty Mass Spectrometry Center).

Jiaying Han progressed in 2018 by testing free and cisplatin complexed cRGfD bioconjuagted Pd_2L_4 metallacage toxicity in cell lines and rat kidney and liver precision cut tissue slices. This study showed cage formation and encapsulation of cisplatin by NMR spectroscopy. Upon encapsulation, cisplatin showed increased cytotoxicity in melanoma A375 cells overexpressing $\alpha v\beta 3$ integrins. Moreover, studies in tissue slices indicated reduced toxicity toward healthy rat precision cut liver and kidney tissues for cage-encapsulated cisplatin. Analysis of metal content by ICP-MS demonstrated that the encapsulated drug is less accumulated in these organs compared to “free” cisplatin. Jiaying Han plans to further characterize the photocleavable properties of a ruthenium complex aimed at the targeted analysis of proteins using mass spectrometry imaging. Jiaying Han is finalizing her thesis to be defended in the first part of 2019.



Plots A and B: Viability of Precision Cut Tissue Slices (PCTS) from liver (A) and kidney (B), treated for 24 h with different concentrations of cisplatin (5, 10 and 25 µM), cage **Co** (10, 30 and 50 µM) and encapsulated cisplatin [cisplatin⊂(**Co**)] ([cage]/[cisplatin] = 5/10, 15/30, 25/50 µM). C and D: Total metal content (Pd and Pt) determined by ICP-MS in PCTS of liver (C) and kidney (D) treated for 24 h with different concentrations of cisplatin, cage **Co** and encapsulated cisplatin ([cisplatin⊂(**Co**)]). The error bars show the standard deviation of at least three independent experiments. For statistical analysis the Two Independent Sample t-Test was applied. * ($p \leq 0.01$) indicates the difference is significant when compared to its control (treatment with cisplatin at the same concentration).[4]

3.2 RNA-based therapy for the treatment of gastrointestinal diseases

Genetic diseases are a major burden in paediatric gastroenterology because of a frequent lack of effective treatments. In particular nonsense mutations, responsible for about 10% of all hereditary gastrointestinal diseases, are among the diseases for which there is no treatment available. Translation read-through-inducing drugs (TRIDs), including aminoglycosides, have been identified as agents, which can suppress pathogenic nonsense mutations and thereby stimulate the production of a complete and functional protein. The goal of this project is to identify the molecular mechanisms of orally administered TRIDs and identify beneficial and health impairing effects of this type of drug. Li Qinghong (PhD student) works on this project with co-supervision by Sven Ijzendoorn (Center for Liver, Digestive & Metabolic Diseases, UMCG) and Victor Guryev (Laboratory of Genome Structure and Ageing, ERIBA, UMCG).

3.3 A chemoproteomic approach to study advanced glycation end-products

Glycolysis is one of the fundamental molecular cell processes, and dysfunctioning of this process leads to uncontrolled glycation of, among others, proteins. Glycation-altered proteins are involved in multiple complex diseases such as cancer and COPD. In this project we aim to develop a novel chemical tool and bioinformatics approach that identifies and quantifies advanced glycation end (AGE)-products of proteins produced by reaction with methylglyoxal at endogenously relevant concentrations. This project was recently funded by the Faculty Theme "Molecular Life and Health" and is a joint project with Martin Witte, leader of the Chemical Biology research group at the Stratingh Institute (RUG).

—

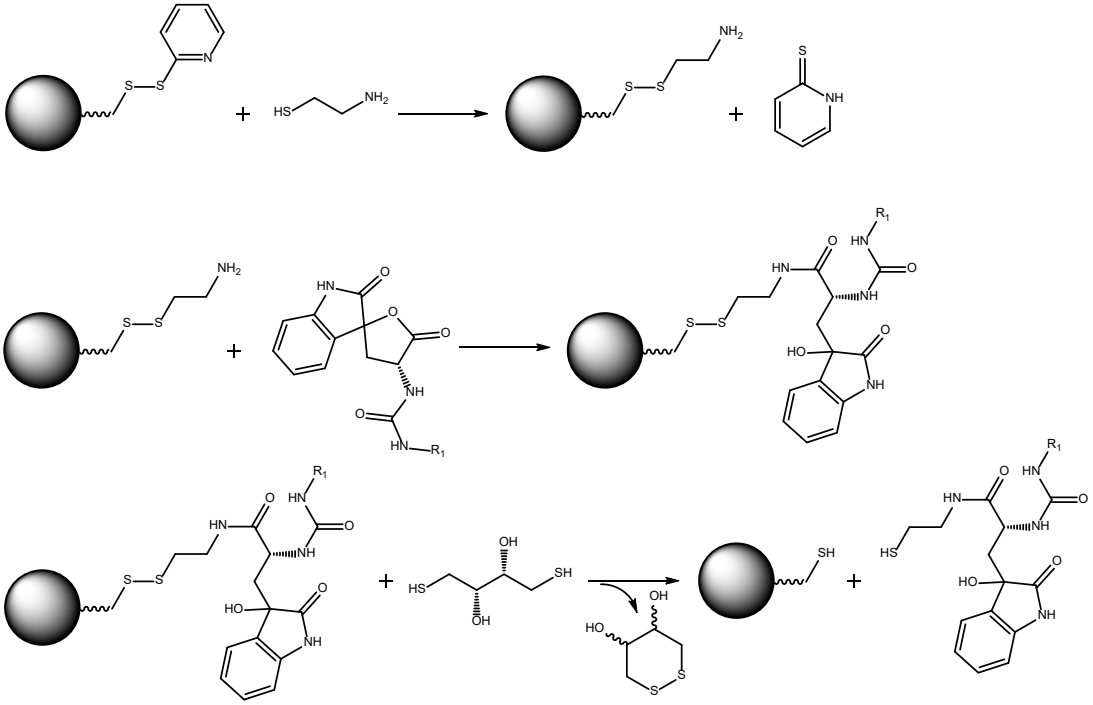
[4] Han et al., Bioconjug Chem 2018, 29, 3856-3865.

4. Electrochemistry-Mass Spectrometry

The different research lines of this project are run in close collaboration between the Analytical Biochemistry Group, the Interfaculty Mass Spectrometry Center (Hjalmar Permentier) and the BIOS Lab-on-a-chip group at Twente University (Mathieu Odijk, Wouter Olthuis, Albert van den Berg). The major topics of the project are the electrochemical conversion of drug molecules into metabolites and the electrochemical cleavage of peptides and proteins.

4.1 Electrochemical peptide bond cleavage

Work on the electrochemical cleavage of peptides and proteins focussed on developing reversible capture-release chemistry to enrich the spirolactone-containing N-terminal cleavage products from the rather complex reaction mixture. Xiaobo Tian (PhD student) further worked on establishing standardized cleavage conditions and on studying critical reaction parameters. He is now moving towards applying these conditions to more complex protein mixtures. Developments of a microfluidics platform to study the reaction in greater detail by spectroelectrochemistry with the goal to provide mechanistic insights into the reaction mechanism (this applies also to project 3.2 below) are ongoing in close collaboration with Mathieu Odijk and Pascal Führer (PhD student) of the BIOS group at Twente University.



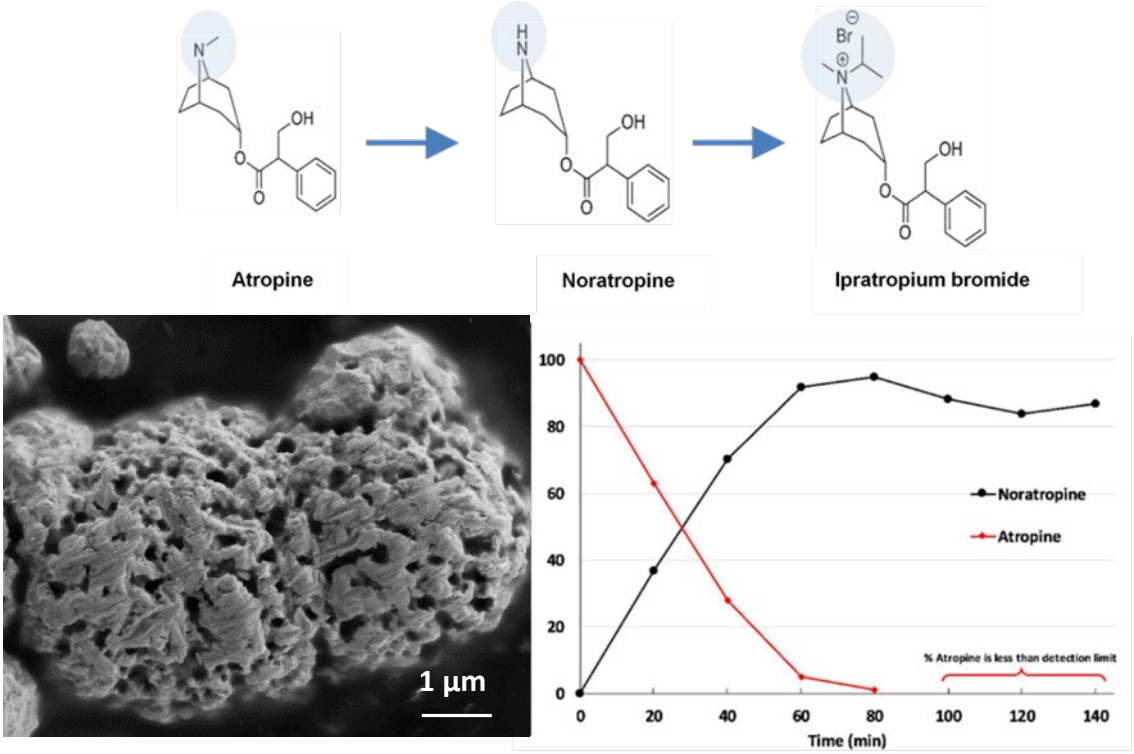
Schematic drawing of a capture and release approach to enrich spirolactone-containing peptides from complex reaction mixtures after electrochemical peptide bond cleavage.

4.2 Electrochemical/catalytic conversion of drug molecules

Nanoporous gold (NPG) surfaces have unusual reactivity and we found that NPG catalyses N-dealkylation reactions of drug compounds even in the absence of an electric potential. Ali Alipour (PhD student) and Jos Hermans (research technician) are continuing the investigations of NPG, focussing respectively on using various techniques to reproducibly prepare and characterize NPG surfaces by alloy mixing (collaboration with Arne Wittstock, University of Bremen), electrodeposition or sputtering (collaboration with Twente University). The aim is to make flow-through reactors in the

form of electrochemical flow-through cells or columns. We are collaborating with the University of Twente (Pascal Führer and Mathieu Odijk) on preparing sputtered NPG surfaces to be used in (EC) flow cells and are planning the design of a spectroelectrochemical device which would help studying the electrochemical reaction mechanisms.

In parallel to the NPG studies, Ali has revisited the optimization of electrochemical parameters for the N-dealkylation reaction, previously studied by Turan Gül (former PhD student). We have chosen tropane alkaloids as substrates, which are of significant interest for the pharmaceutical industry, since they are used as precursors for synthetic drugs, such as the bronchodilator ipratropium bromide. After parameter optimization in an EC flow-cell, the reaction was performed on a larger scale in a batch cell with high yield (~85%) and selectivity. Other tropane alkaloids show similar reactivity. The reaction conditions are much milder than other published methods used to produce nortropanes. Future work will focus on studying these reactions with a wider range of substrate classes as well as in EC flow and batch cells with the NPG electrodes described above.

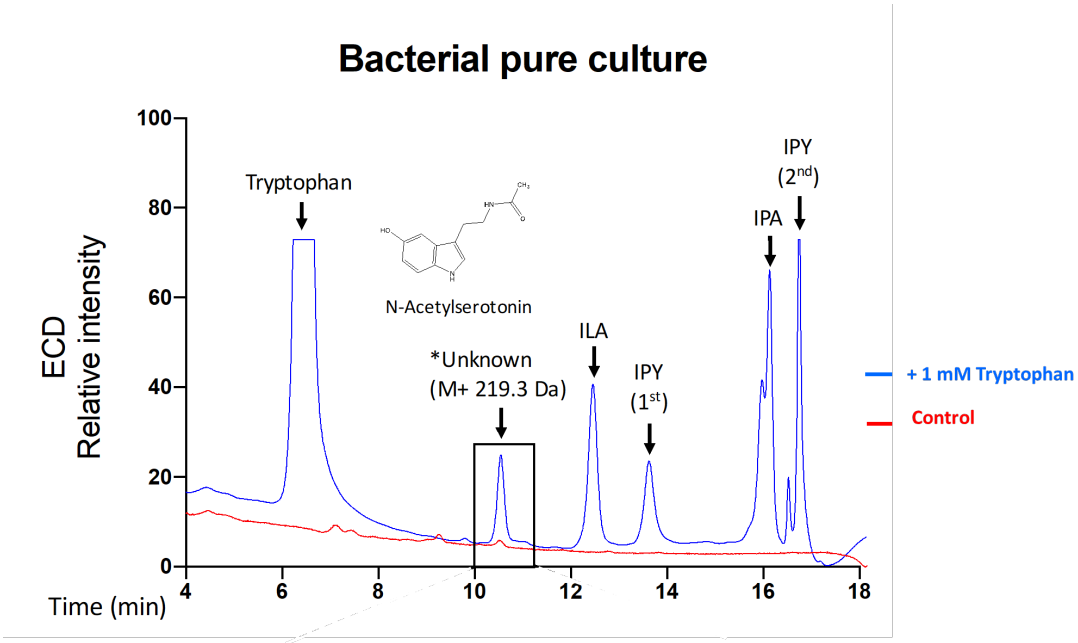


Schematic representation of the N-dealkylation reaction of atropine to noratropine, which is a precursor for the preparation of the bronchodilator ipratropium bromide (top). SEM image of nanoporous gold (NPG) produced by electrochemical deposition of Au and Ag (3:7 molar ratio) followed by nitric acid etching to remove Ag (lower-left). Conversion of atropine to noratropine by electrocatalysis as assessed by LC-MS (lower-right).

4.3. Electrochemical-mass spectrometric detection of neuroactive metabolites produced by gut bacteria

In collaboration with the Microbial Physiology group (Sahar el Aidy) Julia Aresti has started in 2018 as a PhD student in the Molecular Life and Health programme. She is studying the production and transformation of neuroactive compounds in the gut by microbiota, and their effect on the host. The compounds include endogenous and nutritional compounds such as the amino acids tryptophan and tyrosine, and pharmaceutical drugs such as L-DOPA or Ritalin. Many neurotransmitter compounds are electroactive and are analysed with electrochemical detection. Combining ECD with MS in an LC-ECD-MS system allows direct identification of novel metabolites. We have

shown the usefulness of this system for analzying the metabolites of tryptophan and L-DOPA, allowing the identification of compounds such as N-acetylserotonin upon incubation of tryptophan in a gut bacterial culture (see figure) and m-hydroxyphenylacetic acid produced in a rat ceca from L-DOPA-fed animals.

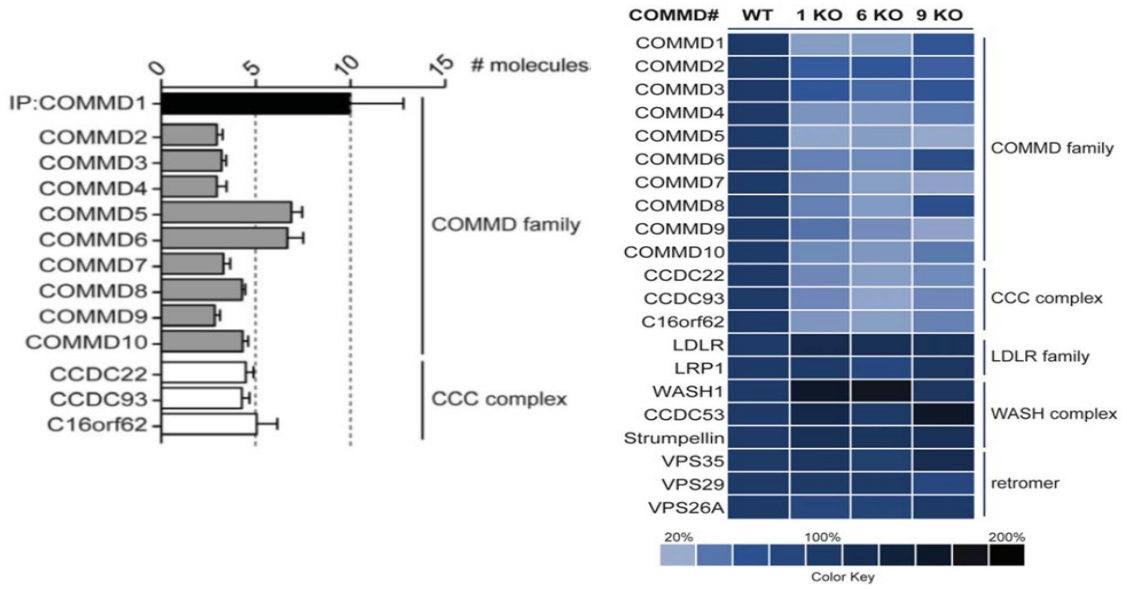


LC-ECD-MS analysis of culture medium of *Clostridium sporogenes* grown in the presence of tryptophan, producing N-acetylserotonin as neuroactive metabolite.

5. Proteomics by targeted mass spectrometry

This research line is driven by Karin Wolters, assisted by Ydwine van der Veen and more and more researchers that connect to Karin to develop targeted LC-MS/MS assays for their respective projects. The use of isotopically labelled internal standards in the form of synthetic concatemers created by the combination of all targeted peptides into one synthetic protein (QconCAT technology) has proven to be of great value to a range of projects. Both, the selected reaction monitoring (SRM) as well as the parallel reaction monitoring (PRM) approach are being pursued, with the SRM approach still representing the 'workhorse'. We currently apply these methods to protein targets related to cellular cholesterol homeostasis and metabolism, triglyceride hydrolysis and atherosclerosis (coll. Kuivenhoven), protein classes like the copper metabolism MURR1 domain (COMMD) protein family (coll. van de Sluis), protein targets related to bile acid metabolism (coll. Kuipers) and mitochondrial plus glycolysis proteins (coll. Bakker).

In the collaborative project with the group of Bart van de Sluis, the COMMD-protein family and connected protein complexes were studied yielding insight in protein complex formation. The figure below shows the quantification of the protein complex after immunoprecipation of one member (left panel) and how the protein complex is perturbed upon knockout of just a single family member (right panel).



Quantification of the COMMD family members in primary hepatocytes after immunoprecipation with COMMD1 (left panel) and perturbation of the protein stability in the complete COMMD family upon k.o. of hepatic COMMD1, 6 or 9 in liver tissues.[5]

In the collaborative project with the group of Jan Albert Kuivenhoven, Antoine Rimbert (Postdoc MolGen) and Wenxuan Zhang (PhD student focusing on lipidomics applications within the Pediatrics Department of the UMCG), we are currently exploring the possibilities to integrate the results from lipidomics, targeted genomics and targeted proteomics in a pilot study focused on individuals from the LifeLines cohort with extreme cholesterol levels.

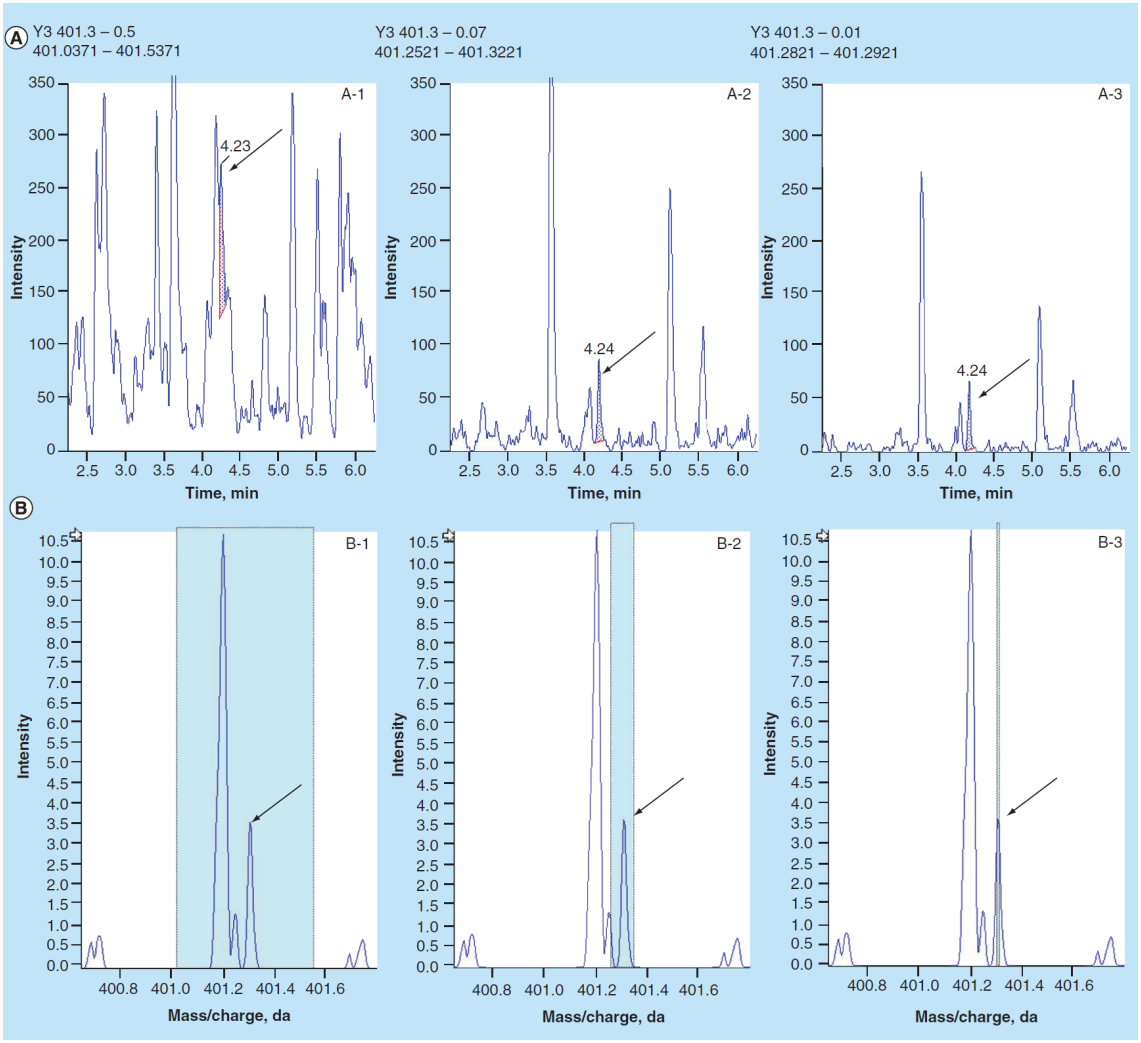
6. Biopharmaceuticals

Our activities in the area of biopharmaceuticals continued in 2018 in collaboration with Nico van de Merbel (PRA Health Sciences) as part of the PhD project of Peter Bults focusing on high-resolution mass spectrometry in quantitative protein bioanalysis. We further extended our work on the biotransformation of Trastuzumab and Pertuzumab as part of the EU-funded ITN network project A4B (11 full partners from 7 countries and 6 associated partners) with 2 PhD students (Oladapo Olaleye and Baubek Spanov) that started in 2018. We recently established a collaboration with the National Cancer Institute (NKI, Amsterdam) to study the *in vivo* biotransformation of Trastuzumab and Pertuzumab in patients undergoing combination therapy for breast cancer with the goal to connect *in vivo* biotransformation to therapeutic efficacy.

The research line focusing on the quantitation of biopharmaceuticals by LC-MS/MS was continued by investigating the potential of LC coupled to high-resolution mass spectrometry (HRMS) as an alternative to the more frequently used combination of LC with triple-quadrupole (MS/MS) detection. In collaboration with the pharmaceutical company Ferring, we developed an LC-HRMS (Q-TOF mass analyser) approach to quantify recombinant human growth hormone (rhGH, somatropin) in plasma without digestion at the whole protein level after immunoaffinity enrichment. The results show that rhGH can be quantified with a sensitivity that surpasses the widely used signature-peptide-based approach and selected reaction monitoring (SRM) on a triple quadrupole mass analyser. This method has been validated according to current international guidelines for application in preclinical PK/PD studies.

—

[5] Fedoseienko et al., Circ. Res., 2018, 122, 1648-1660.



Extracted ion chromatograms for signature peptide SNLELLR after digestion of rat plasma spiked with somatropin at 25 ng/ml (A) and corresponding product ion mass spectra (m/z 401.2871 ($y3^+$)) at the retention time of the analyte (B); mass extraction window of 0.5 Da (A-1, B-1), 0.07 Da (A-2, B-2) and 0.01 Da (A-3, B-3). Arrows indicate retention time (A) or m/z (B) of the analyte. Precursor ion: m/z 422.8.[6]

7. Macrophage polarization in inflammation – the regulatory role of the proteome, protein acetylation and energy metabolism

This research line is driven by Marcel Kwiatkowski (Marie Curier postdoctoral fellow, RESPIRE 3) and the PhD students Alienke van Pijkeren (2+2 PhD student) and Sara Russo (PROMINENT PhD student) in close collaboration with Barbro Melgert and Frank Dekkert (both GRIP). We investigate how macrophage polarization and inflammatory responses in chronic inflammatory diseases, like COPD, obesity and typ-2 diabetes (T2D), are regulated by the proteome - especially by protein acetylation - and energy metabolism (glycolysis and TCA cycle).

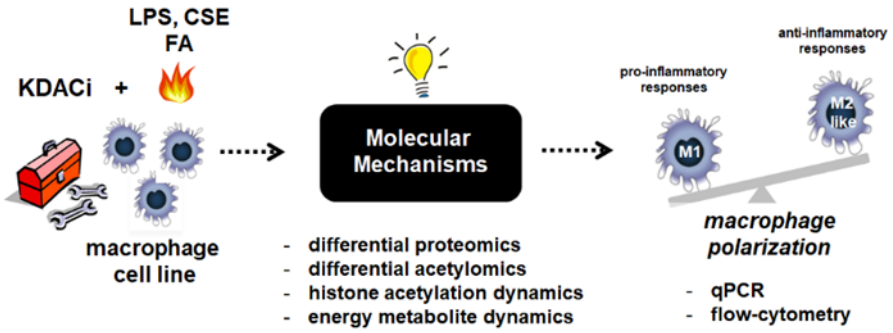
Macrophages are important cells of the immune system that regulate inflammation. They can adopt a variety of different phenotypes (macrophage polarization), depending on the signals they receive from their environment. Macrophages represent the first line of defence against foreign invaders by producing pro-inflammatory cytokines (e.g. IL-1β, TNFα) and generating reactive oxygen and nitrogen species (M1 macrophages), but they

—

[6] Bults et al., Bioanalysis 2018, 10, 1009-1021.

also play an important role in tissue repair and remodelling (M2 macrophages) as well as for the resolution of inflammation (M2-like macrophages) by producing anti-inflammatory cytokines (e.g. IL-10, TGFb). Chronic inflammatory diseases such as COPD, obesity and T2D are associated with an aberrant macrophage polarization, changes in energy metabolism and pro-inflammatory gene expression, and recent research indicates that there is a close link between energy metabolism and gene expression via acetylation of histones and none-histone proteins. In these chronic inflammatory diseases macrophages continue to promote pro-inflammatory responses and have lost their ability to trigger anti-inflammatory responses.

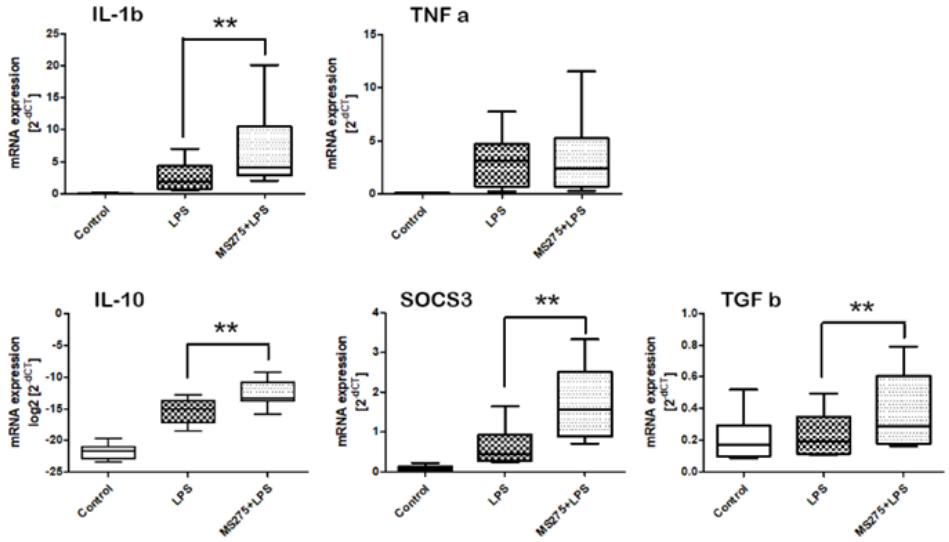
We aim to identify novel molecular mechanisms that drive macrophage polarization from pro-inflammatory responses towards anti-inflammatory responses. For this, we use different macrophage cell lines (MPI macrophages, RAW 264.7 macrophages, primary macrophages). We use different treatments to induce NF-kB mediated inflammation (e.g. LPS, cigarette smoke extract (CSE), high fatty acid concentration) and induce anti-inflammatory macrophage responses by using different lysine deacetylase (KDAC) inhibitors (Figure 1). We analyse pro-inflammatory and anti-inflammatory gene expression and macrophage polarization by using different molecular biological techniques such as qPCR and flow cytometry. To investigate how macrophage polarization and inflammatory gene expression are regulated by changes in the proteome/acetylome and energy metabolism (glycolysis and TCA cycle), we apply and develop different OMICs technologies to analyze the proteome/acetylome, histone acetylation dynamics and dynamics of energy metabolites.



Scheme of the research concept. LPS: lipopolysaccharide, CSE: cigarette smoke extract, FA: fatty acids

7.1. Macrophage polarization in NF-kB mediated inflammation in COPD

Recent results of our research showed that class I KDAC inhibitors induce a shift in macrophage polarization from pro-inflammatory responses towards anti-inflammatory responses in alveolar macrophages (MPI macrophages) in a model system for NF-κB mediated inflammation in COPD. Macrophages were treated with LPS or cigarette smoke extract with and without different KDAC inhibitors. The KDAC inhibitor MS-275 resulted in a significantly upregulation of the anti-inflammatory cytokines IL-10 and TGFb. MS-275 further significantly increased the expression of suppressor of cytokine signaling 3 (SOCS3) which is induced by IL-10. Currently, we are investigating macrophage polarization by flow cytometry and we are analyzing changes within the proteome/acetylome by differential proteomics. In the future, we want to investigate how macrophage polarization correlates with changes in energy metabolites (glycolysis, TCA cycle, e.g. acetyl-CoA, citrate, succinate) and the corresponding metabolic enzymes.

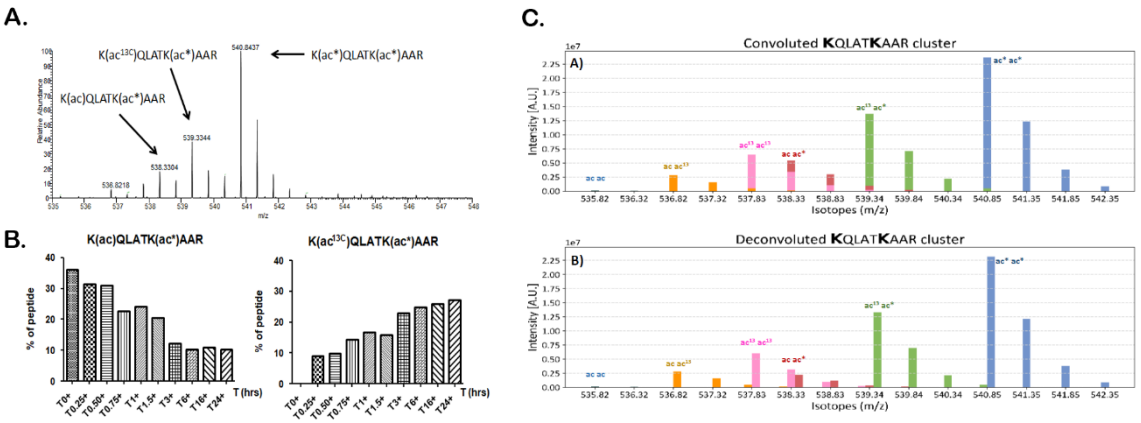


mRNA expression levels in alveolar macrophages (MPI macrophages). Cells were incubated with (MS275+LPS) and without MS-275 (control, LPS) for 16 h. After 16 h cells were stimulated with LPS (c= 10 ng/mL) (LPS, MS275+LPS). The control samples were not incubated with either MS-275 or with LPS. Ct values are expressed as 2 – dCt for visual presentation. For IL-10 the Ct values were log2 transformed. Groups were compared using a Wilcoxon signed-rank test (two-tailed), **p<0.01 was considered significant. n=8. Unpublished data.

7.2. Histone acetylation dynamics in macrophage polarization and inflammation in COPD

In her PhD project, Alienke van Pijkeren develops a methodology to investigate histone acetylation/deacetylation and metabolite dynamics, and correlate them with changes in inflammatory gene expression. The method is based on a combination of metabolic and chemical labeling using stable isotope labeled molecules in cell culture. Following the successful development of a quantitative method for the chemical acetylation of lysine residues using stable isotope-labeled acetic anhydride, Alienke has established a method to study the incorporation of newly synthesized acetyllysine residues into different histone species in an alveolar macrophage cell line using 13C6 glucose as a tracer molecule. Alienke is currently expanding this method with flux analysis of metabolites of the glycolysis and TCA cycle to capture the dynamics of energy metabolism on the one hand, and to calculate the acetylation and deacetylation kinetics of different histone species on the other hand. Rien Leuvenink – bachelor student from the Hanzehoogeschool – is currently supporting Alienke by developing a LC-MS method to analyze metabolites from glycolysis and TCA cycle. The method will be finally used to investigate how changes in energy metabolism and histone acetylation dynamics regulate macrophage polarization and inflammatory responses in NF-kB mediated inflammation in COPD.

During this year we were supported by the master student Tim Lijster (Biomedical Sciences). Tim successfully developed an automatic bioinformatics data processing pipeline for the identification and quantification of acetyllysine isotopoloques of different histone species after chemical and metabolic labeling. Tim developed a Python script to link the identification output of MaxQuant (MQ) to the quantization output of TAPP (Threshold Avoiding Proteomics Pipeline). The pipeline allows the detection of acetyllysine isotopologues in the TAPP output that were not identified by MQ, thus identifying all acetyllysine istopologues for a given histone peptide (isotopologue cluster). The pipeline deconvolutes the detected acetyllysine isotopologue clusters and calculates the relative abundance of each acetyllysine isotopologue, thus improving the quantification of the acetylated histone species.



Incorporation of newly synthesized acetyllysine residues into different histone species (A., B.) and deconvolution of the different isotopologues (C.). ac: endogenous acetyllysine, ac13C: acetyllysine with an acetyl-group obtained from 13C-labeled tracer molecule (here 13C6 glucose), ac*: acetyllysine derived by chemical labeling of lysine residues using 13C4D6 acetic anhydride. Unpublished data.

7.3. Regulation of macrophage polarization and inflammation in typ-2 diabetes (T2D) and obesity

In September 2018 Sara Russo joined our "macrophage" team as a PhD student. Her research project focuses on the "regulation of macrophage polarization and inflammation in typ-2 diabetes (T2D) and obesity through energy metabolism and protein acetylation". Sara is currently establishing a macrophage model system for inflammation in T2D and obesity. Sara uses RAW 264.7 macrophages and induces inflammation by treatment with high concentration of palmitate and/or glucose with (anti-inflammatory response) and without (pro-inflammatory response) KDAC inhibitors. Inflammatory macrophage responses and macrophage polarization will be investigated by qPCR and flow cytometry. In the future, Sara will apply differential proteomics/acetylomics, flux analysis of energy metabolites and our method for histone acetylation dynamic analysis to investigate how macrophage polarization and inflammation is regulated by protein acetylation and energy metabolism.

Ph.D. projects

Andres Gil (Colciencias)
The relevance of preanalytical factors in metabolomics and lipidomics research
Promotor: Rainer Bischoff
Defense: September 14, 2018

Frank Klont
Mass spectrometry-based methods for protein biomarker quantification: on the road to clinical implementation
Promotor: Rainer Bischoff
Start: September 2014 (Defense scheduled for February 08, 2019)

Jiaying Han (CSC scholarship)
Multiplex targeted imaging of biomolecules in tissue with high spatial resolution using laser desorption/ionisation mass spectrometry
Promotor: Peter Horvatovich
Start: October 2014

Peter Bults (PRAHS)
Bioanalysis of proteins
Promotor: Nico van de Merbel
Start: January 2015

Wenxuan Zhang (UMCG)
Lipidomics in Systems Medicine
Promotors: Folkert Kuipers & Dirk-Jan Reijngoud
Start: November 2015

Victor Bernal Arzola
Clinical big data for multifactorial diseases: from molecular profiles to precision medicine
Promotor: Peter Horvatovich
Start: July 2016

Yang Zhang
Proteogenomic and targeted metabolomic analysis of ovarian cancer heterogeneity and its contribution to recurrence and therapy resistance
Promotor: Peter Horvatovich
Start: January 2017

Alienke van Pijkeren (UMCG)
Protein acetylation dynamics – Elucidating the connection between energy metabolism and gene expression in age-related inflammatory diseases
Promotor: Rainer Bischoff
Start: September 2017

Xiaobo Tian (CSC scholarship)
Electrochemistry for Protein and Peptide Chemistry
Promotor: Rainer Bischoff
Start: October 2017

Ali Alipour Najmi Iranag
Electrochemistry – Mass Spectrometry in Proteomics and in Drug Metabolite Synthesis
Promotor: Rainer Bischoff
Start: November 2017

Baubek Spanov
Bioanalytical methodology to study the in vivo biotransformation of therapeutic proteins
Promotor: Rainer Bischoff
Start: May 2018

Oladapo Olaleye
Methodology for studying protein species of therapeutic proteins
Promotor: Rainer Bischoff
Start: June 2018

Alejandro Sánchez Brotons
Development of a generic framework for pre-processing LC/GC-MS(/MS) data obtained with data dependent and independent acquisition
Promotor: Péter Horvatovich
Start: June 2018

Saskia Sokoliova
A chemoproteomic approach to study advanced glycation end-products
Promotor: Péter Horvatovich
Start: July 2018

Sara Russo
Regulation of macrophage polarization and inflammation in Diabetes Mellitus Type II (DMT-II) and obesity through energy metabolism and protein acetylation
Promotor: Rainer Bischoff
Start: September 2018

Janine Stam
Determining exosomal proteins as potential biomarkers for drug-induced cholestasis
Promotor: Rainer Bischoff
Start: October 2018

Julia Aresti Sanz
Detection and characterization of novel metabolites from the gut microbiota with liquid chromatography-electrochemistry-mass-spectrometry, and identification of their biological function
Promotor: Sahar el Aidy
Start: April 2018

Theses

Gil Quintero, J.A., The relevance of preanalytical factors in metabolomics and lipidomics research. Promotor: Rainer Bischoff, Dissertation University of Groningen, September 14, 2018, 210 pp.

Scientific Output

Scientific publications (peer-reviewed)

1. Abushareeda, W., Tienstra, M., Lommen, A., Blokland, M., Sterk, S., Kraiem, S., Horvatovich, P., Nielen, M., Al-Maadheed, M., Georgakopoulos, C., Comparison of gas chromatography quadrupole time-of-flight and quadrupole orbitrap mass spectrometry in anti-doping analysis: I. Detection of anabolic-androgenic steroids. Rapid Communications in Mass Spectrometry 32 (2018) 2055-2064.
2. Abushareeda, W., Vonaparti, A., Saad, K. Al, Almansoori, M., Meloug, M., Saleh, A., Aguilera, R., Angelis, Y., Horvatovich, P.L., Lommen, A.,Alsayrafi, M., Georgakopoulos, C., High resolution full scan liquid chromatography mass spectrometry comprehensive screening in sports antidoping urine analysis. Journal of Pharmaceutical and Biomedical Analysis 151 (2018) 10-24.
3. Al-Thani, A.M., Voss, S.C., Al-Menhali, A.S., Barcaru, A., Horvatovich, P., Al-Jaber, H., Nikolovski, Z., Latiff, A., Georgakopoulos, C., Merenkov, Z., Segura, J., Alsayrafi, M., Jaganjac, M., Whole blood storage in CPDA1 blood bags alters erythrocyte membrane proteome. Oxidative Medicine and Cellular Longevity (2018), https://doi.org/10.1155/2018/6375379.
4. Bronsema, K.J., Klont, F., Schalk, F.B., Bischoff, R., Kema, I.P., Merbel, N.C. van de, A quantitative LC-MS/MS method for insulin-like growth factor 1 in human plasma. Clinical chemistry and laboratory medicine 56 (2018) 1905-1912.
5. Bults, P., Meints, M., Sonesson, A., Knutsson, M., Bischoff, R., Merbel, N.C. van de, Improving selectivity and sensitivity of protein quantitation by LC-HR-MS/MS: Determination of somatropin in rat plasma. Bioanalysis 10 (2018) 1009-1021.
6. Cheng, D., Qiao, L., Horvatovich, P., Toward spectral library-free matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry bacterial identification. Journal of Proteome Research 17 (2018) 2124-2130.
7. Gil, A., Zhang, W., Wolters, J.C., Permentier, H., Boer, T. de, Horvatovich, P., Heiner-Fokkema, M.R., Reijngoud, D.J., Bischoff, R., One- vs two-phase extraction: re-evaluation of sample preparation procedures for untargeted lipidomics in plasma samples. Analytical and Bioanalytical Chemistry 410 (2018) 5859-5870.
8. Güzel, C., Govorukhina, N.I., Stingl, C.,Dekker, L.J.M., Boichenko, A., Zee, A.G.J. van der, Bischoff, R., Luider, T.M., Comparison of targeted mass spectrometry techniques with an immunoassay : A Case Study For HSP90α. Proteomics. Clinical Applications, 12 (2018) 1700107.
9. Güzel, C., Govorukhina, N., Wisman, G.B.A., Stingl, C., Dekker, L., Hollema, H.,Guryev, V., Horvatovich, P., Zee,A. van der, Bischoff, R., Luider, T., Proteomic alterations in early stage cervical cancer. Oncotarget 9 (2018) 18128-18147.
10. Han, J.; Rader, A.; Reichart, F.; Aikman, B.; Wenzel, M.N.; Woods, B.; Weinmüller, M.; Ludwig, B.S.; Sturup, S.; Groothuis, G.M.M.; Permentier, H.P.; Bischoff, R.; Kessler, H.; Horvatovich, P.; Casini, A.. Bioconjugation of Supramolecular Metallacages to Integrin Ligands for Targeted Delivery of Cisplatin. Bioconjugate Chemistry, 29 (2018) 3856-3865.
11. Han, J.; Permentier, H.; Bischoff, R.; Groothuis, G.; Casini, A.; Horvatovich, P., Imaging of protein distribution in tissues using mass spectrometry: an interdisciplinary challenge. TRAC – Trends in Analytical Chemistry, (Dec. 21, 2018; just accepted).
12. Klont, F., Pouwels, S., Hermans, J., Merbel, N.C. van de, Horvatovich, P., Hacken, N.H.T. ten, Bischoff, R., A fully validated liquid chromatography-mass spectrometry method for the quantification of the soluble receptor of advanced glycation end-products (sRAGE) in serum using immunopurification in a 96-well plate format. Talanta 182 (2018) 414-421.
13. Klont, F., Hadderingh, M., Horvatovich, P., Hacken, ten Hacken, N.H.T., Bischoff, R., Affimers as an alternative to antibodies in an affinity LC-MS assay for

quantification of the soluble receptor of advanced glycation end-products (sRAGE) in human serum. Journal of Proteome Research 17 (2018) 2892-2899.
14. Klont, F.; Bras, L.; Wolters, J.C.; Ongay, S.; Bischoff, R.; Halmos, G.B.; Horvatovich, P. Assessment of sample preparation bias in mass spectrometry-based proteomics. Analytical Chemistry 90 (2018) 5405-5413.
15. Klont, F., Joosten, M.R., Hacken, N.H.T. ten, Horvatovich, P., Bischoff, R., Quantification of the soluble Receptor of Advanced Glycation End-Products (sRAGE) by LC-MS after enrichment by strong cation exchange (SCX) solid-phase extraction (SPE) at the protein level. Analytica Chimica Acta 1043 (2018) 45-51.
16. Kwiatkowski, M., Krösser, D., Wurlitzer, M., Steffen, P., Barcaru, A., Krisp, C., Horvatovich, P., Bischoff, R., Schlüter, H., Application of displacement chromatography to online two-dimensional liquid chromatography coupled to tandem mass spectrometry improves peptide separation efficiency and detectability for the analysis of complex proteomes. Analytical Chemistry 90 (2018) 9951-9958.
17. Mitra, V., Smilde, A.K., Bischoff, R., Horvatovich, P.,Tutorial : Correction of shifts in single-stage LC-MS(/MS) data. Analytica Chimica Acta 999 (2018) 37-53.
18. Ongay, S., Langelaar-Makkinje, M., Stoop, M.P., Liu, N., Overkleeft, H.,Luider, T.M., Groothuis, G.M.M., Bischoff, R., Cleavable crosslinkers as tissue fixation reagents for proteomic analysis. ChemBioChem 19 (2018) 736-743.
19. Perez Montoro, B., Benomar, N., Gómez, N.C., Ennahar, S., Horvatovich, P., Knapp, C.W., Gálvez, A., Abriouel, H., Proteomic analysis of Lactobacillus pentosus for the identification of potential markers involved in acid resistance and their influence on other probiotic features. Food Microbiology 72 (2018) 31-38.
20. Pérez Montoro, B., Benomar, N., Caballero Gómez, N., Ennahar, S., Horvatovich, P., Knapp, C.W., Alonso, E., Gálvez, A., Abriouel, H., Proteomic analysis of Lactobacillus pentosus for the identification of potential markers of adhesion and other probiotic features. Food Research International 111 (2018) 58-66.

Book chapters

1. Gil, A.; Wenxuan Zhang, W.; Wolters, J.C.; Permentier, H.P.; Horvatovich, P.L.; Heiner-Fokkema, M.R.; Reijngoud, D.J.; Bischoff, R.; Omics Technology: lipidomics and its pitfalls during the pre-analytical stage. Encyclopedia of Analytical Science, 3rd Edition; Elsevier Ltd., Oxford, UK, 2018; doi.org/10.1016/B978-0-12-409547-2.14002-8.
2. Klont, F.; Horvatovich, P.L.; Govorukhina, N.I.; Bischoff, R.: Pre- and postanalytical factors in biomarker discovery; Proteomics for Biomarker Discovery. In Methods in Molecular Biology (eds. Bruin, V. & Couté, B.), Springer Nature, 2018; submitted.
3. Horvatovich, P.L., Brandsma, C.-A.; Suits, F.; Bischoff, R.; Victor Guryev, V.; Proteogenomics and multi-omics data integration for personalized medicine. Handbook of Biomarkers and Personalized Medicine (eds. Carini C., Fidock M., van Gool A.), CRC Press; 2018; submitted.
4. Wolters, J.C.; Permentier, H.P.; Bakker, B.M.; Bischoff, R.: Targeted proteomics to study mitochondrial biology. In Advances in Experimental Medicine and Biology, (eds. Babu M., Urbani A.); Springer Nature, 2018; submitted.
5. Merbel, N. van de, Sample preparation for quantitative LC-MS bioanalysis of proteins. In: Sample Preparation in LC-MS Bioanalysis, (eds. Li W., Zhang J., and Tse F.L.S.), John Wiley and Sons; 2018, in press.

Other publications

1. Bischoff, R., Developing novel methods for protein, peptide and metabolite analysis. Bioanalysis 10 (2018) 431-432.
2. Klont F., Demoralization and stress—we can all help (?). Occupational Medicine 68 (2018) 17.

Lectures

1. Bischoff, R., Biomarker discovery and validation – from shotgun proteomics to targeted methods. ISPPP2018, Berlin, Germany, November 04, 2018 (keynote lecture)
2. Bischoff, R., Quantitative bioanalysis of proteins by LC-MS. MSB2018, Rio de Janeiro, Brazil, February 19, 2018 (keynote lecture)
3. Bischoff, R., Electrochemistry combined with mass spectrometry – fundamentals and applications in protein chemistry and drug metabolism research. Sao Carlos Federal University, Sao Carlos, Brazil, February 28, 2018
4. Bischoff, R., Biomarker discovery and validation – from shotgun proteomics to targeted methods. 12th CEEPC, Bucharest, Romania, October 25, 2018 (keynote lecture)
5. Bischoff, R., Quantification of proteins in complex biological samples by LC-MS. PBA2018, Leuven, Belgium, September 12, 2018 (keynote lecture)
6. Bischoff, R., Electrochemical, site-specific peptide bond cleavage as a novel approach in protein analysis. KNCV Working Group Electrochemistry, Groningen, The Netherlands, November 23, 2018
7. Bults, P., Liquid chromatography – high-resolution mass spectrometry for the quantification of recombinant human growth hormone. University of Strasbourg, Faculty of Pharmacy, Strasbourg, France, June 19, 2018
8. Horvatovich, P., Exploring the limits of mass spectrometry molecular profiling for clinical applications, PIL lecture, University of Groningen, Groningen, The Netherlands, February 13, 2018
9. Horvatovich, P., Exploring the limits of mass spectrometry molecular profiling for clinical applications, Casa Foundation, University of Amsterdam, Amsterdam, The Netherlands, May 7, 2018
10. Horvatovich, P., Mass spectrometry data structure and interpretation for proteomics and metabolomics, “Human Proteome Organization, Bioinformatics resources”, and “Proteogenomics to reveal the molecular mechanism of COPD”, DTL programmer meeting, Utrecht, The Netherlands, March 16, 2018
11. Horvatovich, P., Assessment of sample preparation bias in mass spectrometry-based proteomic, 19th C-HPP Workshop, Santiago de Compostela, Spain, June 16, 2018
12. Klont, F., Massaspectrometrische alternatieven voor IGF1 kwantificering (in de kliniek). NVKC Clinical Mass Spectrometry section symposium, Arnhem, The Netherlands, March 13, 2018
13. Klont, F., Low to sub ng/mL quantification of the soluble receptor for advanced glycation end-products (sRAGE) in human serum using UPLC-MRM-MS. Waters Protein & Peptide MS Bioanalysis Workshop, Eschborn, Germany, October 30, 2018
14. Klont, F., Low to sub ng/mL quantification of the soluble receptor for advanced glycation end-products (sRAGE) in human serum using UPLC-MRM-MS. NVMS Fall Meeting 2018, Amsterdam, The Netherlands, November 15, 2018
15. Kwiatkowski, M., Displacement chromatography mode – High protein sequence coverages and identification rates for low µg-range proteomics using online 2D-LC-MS. European Mass Spectrometry Conference (EMSC), Saarbrücken, Germany, March 15, 2018
16. Merbel, N.C. van de, Finding the root cause for very complex IS variation impacting data reliability and accuracy. 12th Workshop on Recent Issues in Bioanalysis, Philadelphia, PA, USA, April 12, 2018.
17. Merbel, N.C. van de, Quantitation of (monoclonal) antibodies in biological samples by LBA and/or LC-MS - current state of the art. Ferring Pharmaceuticals monoclonal antibody development workshop, Copenhagen, Denmark, May 4, 2018.

18. Merbel, N.C. van de, And what happens to our well-characterized products? In vivo biotransformation of biopharmaceuticals. FABIAN, Nijmegen, the Netherlands, October 5, 2018
19. Merbel, N.C. van de, Instability of biological matrices and its effect on bioanalytical method performance. European Bioanalysis Forum, 11th Open Symposium, Barcelona, Spain, November 22, 2018
20. Permentier, H.P., Analysis of drug metabolites generated by electrochemistry coupled to mass spectrometry. NVMS Spring Meeting, Nieuwegein, The Netherlands, April 12, 2018

Poster presentations

1. Klont, F., Pouwels, S.D., Hadderingh, M., Joosten, M.R., Hermans, J., Merbel, N.C. van de, Horvatovich, P., Hacken, N.H.T. ten, Bischoff, R., Antibody-free LC-MS methods for low to sub ng/mL quantification of the soluble Receptor for Advanced Glycation End-products (sRAGE) in serum. International Mass Spectrometry Conference (IMSC) 2018, Florence, Italy, August 26-31, 2018
2. Kwiatkowski, M., Schlueter, H., Cold vaporization of tissues by Picosecond Infrared Laser Ablation (PIRL) - Unique access to the original protein composition. International Mass Spectrometry Conference (IMSC) 2018, Florence, Italy, August 26-31, 2018
3. Tian, X., Zhang, T., Permentier, H., Bischoff, R., Enrichment of electrochemically cleaved peptides for middle-down proteomics applications. CHAINS 2018, Veldhoven, The Netherlands, December 3-5, 2018
4. Zhang, T., Tian, X., Permentier, H., Bischoff, R., Enrichment of electrochemically cleaved peptides for middle-down proteomics applications. International Mass Spectrometry Conference (IMSC) 2018, Florence, Italy, August 26-31, 2018
5. van Pijkeren, A., Kwiatkowski, M., Bischoff, R., Chemical acetylation of histone lysine residues at the protein level using acetic anhydride. International Mass Spectrometry Conference (IMSC) 2018, Florence, Italy, August 26-31, 2018

Editorships/board memberships

Bischoff, R., Board: Section Analytical Chemistry (KNCV)

Bischoff, R., Board: Dutch Proteomics Platform

Horvatovich, P., Secretary General of C-HPP

Horvatovich, P., PI - Chromosome 5 (C-HPP)

Merbel, N.C van de, Harmonization team leader of the Global Bioanalysis Consortium (GBC)

Merbel, N.C. van de, editorial board member Bioanalysis (Future Science Group).

Merbel, N.C. van de, Topic Team member: European Bioanalysis Forum

Merbel, N.C. van de, Board: Section Analytical Chemistry (KNCV)

Merbel, N.C. van de, Board: Working Group Pharmaceutical and Biomedical Analysis (KNCV)

Prizes/Awards

Kwiatkowski, M., Identification of novel molecular mechanisms regulating inflammation in pulmonary macrophages in COPD using differential acetylomics. Noordelijke CARA Stichting, €4900, The Netherlands.

Kwiatkowski, M., Netherland Respiratory Society (NRS) Young Investors Travel Grant, €1250, The Netherlands.

Press/Media

Bischoff, R., New paper demonstrates Affimer® based assay for the quantification of sRAGE in COPD. 23/07/2018. Introduction to paper in J. Proteome Research in Avacta Newsletter, with reference to publication and blog about this publication. https://www.avacta.com/blogs/validated-clinical-affimer-assay-quantification-srage-copd

Bischoff, R., 15/03/2018. Novel methods for protein, peptide and metabolite analysis: An interview with Rainer Bischoff. Bioanalysis Zone. https://www.bioanalysis-zone.com/2018/03/15/developing-novel-methods-protein-peptide-metabolite-analysis-interview-rainer-bischoff/

Research Grants:

Funded Projects

National Roadmap for Large-Scale Research Infrastructure (NWO 184.034.019)
Netherlands X-omics Initiative
Principal Investigator: Alain van Gool (UMCRadboud, Nijmegen)
Funding Period: 2018-2028

GRIP PhD Scholarship
Recipient: Janine Stam
Determining exosomal proteins as potential biomarkers for drug-induced cholestasis
Principal Investigator: Rainer Bischoff
Funding Period: 2018-2022

Dutch Heart Foundation
High throughput Screening to identify novel molecules enhancing the activity of the CArdio-Protective Enzyme 5-oxoprolinase (OPLAH) for the treatment of Heart Failure. – eSCAPE-HF
Principal Investigator: Peter van der Meer (University Medical Center Groningen)
Funding Period: 2018-2021

Molecular Life Sciences and Health (University of Groningen)
A chemoproteomic approach to study advanced glycation end-products
Principal Investigators: Peter Horvatovich and Martin Witte (Stratingh Institute, University of Groningen)
Funding Period: 2017-2021

Molecular Life Sciences and Health (University of Groningen)
Combining liquid chromatography-electrochemical detection with mass spectroscopy for powerful characterization of novel neuroactive gut bacterial metabolites with potential antimicrobial activity
Principal Investigators: Hjalmar Permentier and Sahar El Aidy (Groningen Biomolecular and Biotechnology Institute (GBB), University of Groningen)
Funding Period: 2017-2021

RESPIRE3 Marie-Curie Fellowship R3201703-00121
“Proteomics-based Pharmacological Biochemistry (P2B2)” - A strategy to identify protein species and signaling pathways regulating inflammatory responses of pulmonary macrophages in NFκB-mediated inflammation and COPD
Recipient: Marcel Kwiatkowski
Funding Period: 2017-2019

H2020-MSCA-ITN-2017; Marie Skłodowska-Curie Innovative Training Network (ITN) - European Training Network (ETN)
Analytics for Biologics (A4B)
Principal Investigator: Hartmut Schlüter (University Medicine Hamburg, Germany)
Funding Period: 2017-2020

H2020-MSCA-COFUND-2016; Marie Skłodowska-Curie Action
‘PROMINENT’ Personalised Medicine in Diabetic Chronic Disease Management
Principal Investigator: Dick de Zeeuw (University Medical Center Groningen)
Funding Period: 2017-2020

NWO-TTW 15230
Nano-patterned Electrochemical Surfaces for Protein Analysis and Drug Synthesis
Principal Investigator: Mathieu Odijk (Twente University, Enschede, The Netherlands)
Funding Period: 2017-2021

EU-COST CA16113
CliniMARK: 'good biomarker practice' to increase the number of clinically validated biomarkers
Principal Investigator: Theo Luider (Erasmus Medical Center, Rotterdam)
Funding Period: 2017-2021

Data System Complexity (University of Groningen) with support from Prof. Dr. Erik Frijlink
Clinical Big Data for multifactorial diseases: from molecular profiles to precision medicine
Principal investigator: Péter Horvatovich
Funding Period: 2016-2020

TRANSCAN-2 ERA-NET TRS-2015-00000149
Proteogenomic and targeted metabolomic analysis of ovarian cancer heterogeneity and its contribution to recurrence and therapy resistance
Principal investigator: Christiane A. Opitz (German Cancer Research Center, DKFZ, Heidelberg, Germany)
Funding Period: 2016-2019

Qatar Research Foundation NPRP8-1472-3-290
Risk Assessment of Mycotoxin Exposure through dietary exposure in Qatar
Principal Investigator: Peter Horvatovich
Funding Period: 2016-2020

Chromosome-Centric Human Proteome Project (C-HPP)
Chair: Young-Ki Paik (Yonsei University, Seoul)
Responsible Scientist for the Chromosome 5 team and Secretary General: Peter Horvatovich
Period: 2012-2022

NWO-STW Perspectief program P12-04
Biomarker Development Center (BDC)
Principal investigator: Rainer Bischoff
Co-investigators:
Theo M. Luider & Arfan Ikram (Erasmus Medical Center, Rotterdam), Alain van Gool & Ron Wevers (Radboud University Medical Center, Nijmegen), Nick ten Hacken (University Medical Center Groningen)
Funding Period: 2014-2019

Teaching

Biotechnology (WLB07045)	Feb. 04 – April 11, 2018
Quantitative Bioanalysis (WMFA14005)	Feb. 05 – 23, 2018
Fatem	Feb. 08 – March 09, 2018
Proteomics/Genomics (WLB07041)	March 19 – April 6, 2018
Master d'Analyse des Médicaments, "Introduction à la protéomique" (in French), University of Strasbourg	March 23, 2018
Bachelor thesis & project (WLFB0812 & WLFB0811)	April 16 - June 22, 2018
Medical Genomics & Proteomics (WLB07090)	May 17 – June 01, 2018
Biostatistics (WLFB1001)	June 05 - 25, 2018
Pharmaceutical Analysis C (WBFA16007)	September 03 - October 12, 2018
MPDI TOP master (MPDI Topclass 1)	September 05, 2018
Drug Development (masters)	Sept. 07, 2018
MPDI TOP master (MPDI Topclass 2)	October 30, 2018
Biomedical Sciences lab tour	November 2, 2018
Academic Research & Communication Skills,	November 6-8, 2018
MPS master, Proteomics applications for personalized medicine	November 16, 2018
Molecular and Cellular Neuroscience (MLBCNN07)	November 21, 2018
Mass Spectrometry (open course)	Nov. 29-30, 2018
MPS Career event, Computational mass spectrometry: an interdisciplinary challenge,	November 29, 2018

Bischoff, R., member of the 'examen commissie' Pharmacy
Bischoff, R., tutor for the master Medical and Pharmaceutical Sciences (MPS)
Horvatovich, P., member of the 'curriculum commissie' Bachelor Pharmacy
Horvatovich, P., 'toelating commissie', MPS
Horvatovich, P., member of the 'opleidingscommissie', Pharmacy

Special teaching activities

Bischoff, R., Analysis of proteins, peptides and metabolites by liquid chromatography-mass spectrometry, master class at Sao Carlos Federal University, Sao Carlos, Brazil, February 26 – March 2, 2018

Bischoff, R., Organization of the A4B Training School 1 "Basics in protein production and bioanalytics" (EU-ITN network), Groningen, The Netherlands, June 11 – 22, 2018
Lecturers a.o. Bischoff, R., Merbel, N.C. van de

Student projects

Marc Joosten, start 14-8-2017: Development of an antibody-free, LC-MS-based quantitative workflow for serum sRAGE on the basis of ion exchange solid phase extraction (MSc project supervised by Frank Klont), end date April 24, 2018

Marrit Hadderingh, start 1-9-2017, Hogeschool VHL Leeuwarden, project: Ontwikkeling en validatie van een immunoaffiniteit-LC-MS methode voor het kwantificeren van sRAGE in serum gebruikmakend van affimeren als

affiniteitsliganden. Bachelor project supervised by Frank Klont, end date January 26, 2018

Naomi Sanders, Development of a immonucapture LC-MS method for the determination of Intact recombinant human growth hormone in rat EDTA plasma. Bachelor project supervised by Peter Bults, end date June 29, 2018.

Hedwich Meindertsma, finished January 2018, Development of a method (ELISA) for the determination of Trastuzumab antibodies in human EDTA plasma samples. Bachelor project supervised by Peter Bults, end date January 10, 2018.

Robin Soemopawiro, Development of a receptor based sandwich ELISA for the determination of pharmacologically active Trastuzumab in human EDTA plasma samples. Master's project supervised by Peter Bults and Natalia Govorukhina, end date May 24, 2018.

Susan Visscher per 1-9-2018, Hogeschool VHL Leeuwarden, project: Isobaric Peptide Termini Labelling for quantification of the proteome. Bachelor project supervised by Xiaobo Tian, estimated final date 25-1-2019.

Jamil Karchoud, Computer Science (FSE, RUG), Visualisation for Threshold-Avoiding Proteomics Pipeline. Bachelor project supervised by Peter Horvatovich, end date September 24, 2018.

Tim Lijster, Biomedical Sciences, Histone acetylation dynamics - Development of an automatic LC-MS/MS data analysis pipeline for the investigation of histone acetylation dynamics using stable isotopes. Master's project supervised by Peter Horvatovich and Marcel Kwiatkowski, end date September 13, 2018.

Rien Leuvenink, per 12-11-2018, Hanzehoogeschool, project: Development of an LC-MS method to quantify metabolites of the glycolysis and TCA cycle and their isotopologues. Bachelor project supervised by Marcel Kwiatkowski

Adelina Dinter, per 03-12-2018, University of Münster (Germany), project: Electrocatalytic/catalytic N-dealkylated metabolite synthesis. Erasmus project supervised by Ali Alipour

Andrea Kurtinović, per 26-11-2018, IMI international master's, project: Bioanalysis of biotransformation products of Trastuzumab and Pertuzumab. Master's project supervised by Baubek Spanov

Outlook

2018 was a year of changes and these changes will radiate into 2019. New PhD students must find their way and get started in their respective projects. This is always an exciting as well as a challenging period for everyone. We do hope that the new generation of PhD students will continue as successfully as the one's that finished or will finish soon. From what we've seen so far, things look promising but it is still early days.

2019 will hopefully also bring the necessary investments in infrastructure from the UMCG and the RUG. While this is still under discussion, investment in a new high-resolution mass spectrometer has been secured through the X-omics grant, which will allow us to advance our work on biopharmaceuticals as well as the area of data-independent acquisition in proteomics and possibly other Omics areas. Data pre-processing and data analysis will form a critical part of this work.

While the biomarker research lines are suffering from a lack of funding, we do hope that grant applications in the areas of proteogenomics and data integration will be awarded to overcome this bottleneck. Peter Horvatovich has deployed a number of activities in these areas and the X-omics grant will allow us to continue in this direction.

Thanks to many dedicated PhD students, postdocs and staff members, 2018 was again a year with good scientific output with 20 peer-reviewed publications and contributions to 5 book chapters. The fact that many of us were asked to give oral presentations is a further reflection of the quality of our work and the recognition that we receive for it. We are confident that this will continue in 2019.

Cooperation between the Analytical Biochemistry Group and the Interfaculty Mass Spectrometry Center is a cornerstone of our successful work. As the department head approaches his 63rd birthday in 2019, Hjalmar Permentier and Peter Horvatovich take on more responsibilities. I am confident that Hjalmar and Peter will do this very well and keep the department and the IMSC running in a sustainable manner based on high-quality scientific and service work. I am happy to have such excellent scientists and managers next to me.

As this is the 16th Annual Report (the first was issued in 2003), I took a look back at all the PhD students and postdocs that passed through the department. I am notably proud of the international atmosphere that we kept over the years. I believe that natural science is one of a few areas where people from all over the world can get along and work together despite different social backgrounds, mother tongues, ethnic backgrounds or religions. We ow much of this to our common understanding of the world through natural science as well as to our *lingua franca* English. While non-native speakers all struggle at times with English speaking or writing, it is thanks to our common efforts that people from all over the world can communicate with each other. I hope it will stay this way in times to come. I placed the flags of the 26 countries, from which coworkers in the group come and came, on the last page.

With this, I would like to thank you for your interest in this report and in our work. Please don't hesitate to contact us if you feel that our expertise and infrastructure could be of interest to one of your ongoing or planned research projects.


Netherlands
Germany
Venezuela
China
Iran
Colombia
Romania
Serbia
France
Ireland
Sweden
Pakistan
Ukraine
Turkey
Poland
Indonesia
Hungary
India
Nigeria
Italy
Slovakia
Russian Federation
Spain
Kazakhstan
Greece
Brazil