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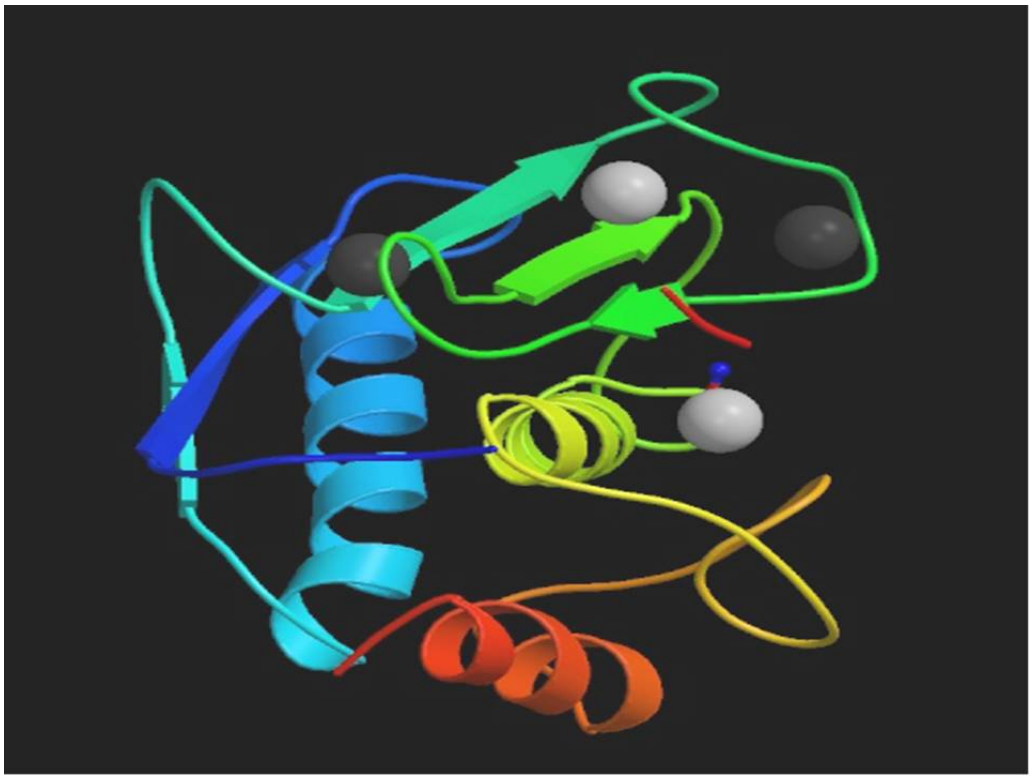
Analytical Biochemistry & Interfaculty MS Center

Annual Report 2019

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

January 09, 2020



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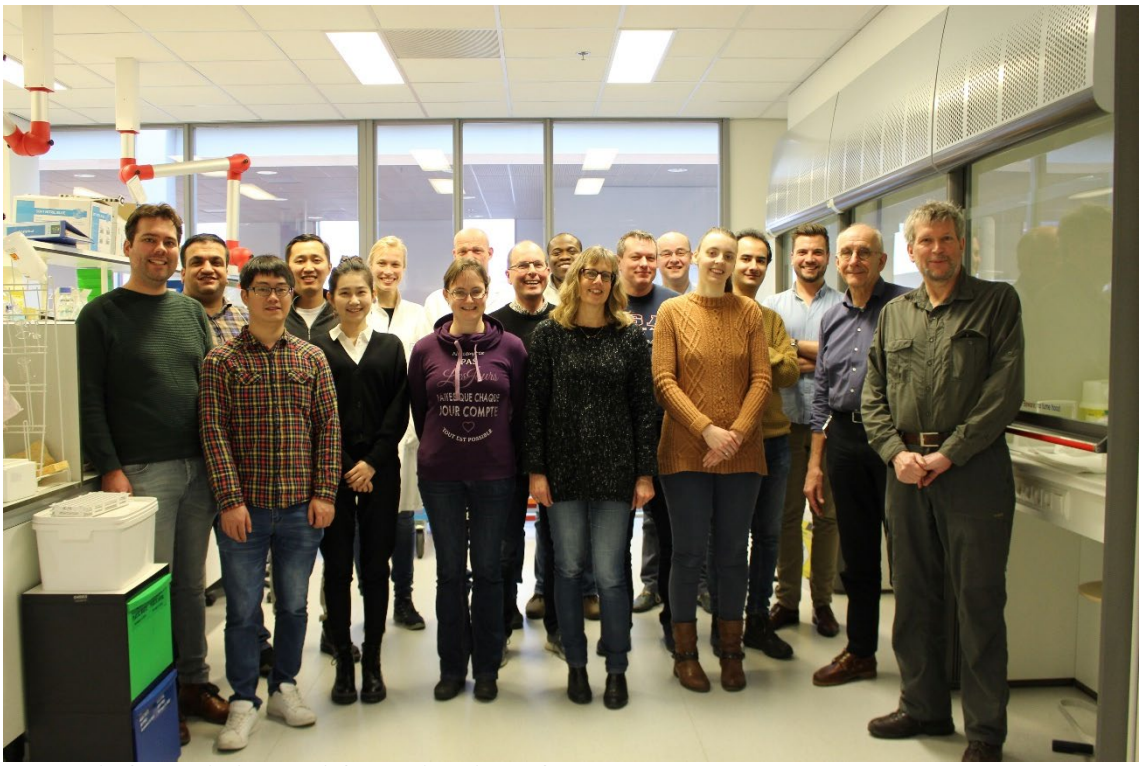

From left to right and from back to front:
Bas Sleumer, Walid Maho, Xiaobo Tian, Baubek Spanov, Wenxuan Zhang, Ydwine van der Veen, Karin Wolters, Marcel de Vries, Nico van de Merbel, Oladapo Olaleye, Jolanda Meindertsma, Peter Horvatovich, Hjalmar Permentier, Janine Stam, Ali Alipour, Rik Beernink, Dirk-Jan Reijngoud, Rainer Bischoff,
Not on photo:
Jos Hermans, Andrei Barcaru, Alejandro Sánchez Brotons, Julia Aresti Sanz, Jan Willem Meints, Natalia Govorukhina, Thomas Cremers, Yang Zhang, Alienke van Pijkeren, Saskia Sokoliova, Sara Russo, Victor Bernal Arzola

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Members of the Research Groups

Staff

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Prof. Dr. Peter Horvatovich (Associate Professor)
Prof. Dr. Nico van de Merbel (by special appointment, PRAHS)
Prof. Dr. Thomas Cremers (by special appointment, CAN Holding)
Dr. Karin Wolters (UMCG)
Dr. Natalia Govorukhina
Jos Hermans
Jan Willem Meints
Jolanda Meindertsma (secretary; 0.4 fte)

Interfaculty Mass Spectrometry Centre (IMSC)

Dr. Hjalmar Permentier (head IMSC)
Marcel de Vries (UMCG)
Walid Maho
Ydwine van der Veen (UMCG)

Post-doctoral researchers

Dr. Andrei Barcaru (UMCG)

Ph.D. students

Frank Klont (thesis defense Feb. 08, 2019)
Jiaying Han (thesis defense scheduled for Feb. 07, 2020)
Peter Bults (PRAHS)
Victor Bernal Arzola
Bas Sleumer (PRAHS)
Wenxuan Zhang (UMCG)
Yang Zhang
Xiaobo Tian
Ali Alipour
Alienke van Pijkeren (UMCG)
Wadha Abushareeda (Qatar Antidoping Lab)
Julia Aresti Sanz (shared PhD with Microbial Physiology, GBB/RUG)
Baubek Spanov
Oladapo Olaleye
Alejandro Sánchez Brotons
Saskia Sokoliova (shared PhD with the Stratingh Institute of Chemistry/RUG)
Sara Russo
Janine Stam
Rik Beernink (IQ Products)

Research Students

Susan Visscher (until January 25, 2019)
Rien Leuvenink (until August 31, 2019)
Adelina Dinter (until March 29, 2019)
Jessica Alferez del Castillo (start November 18, 2019)
Helmut Stanzl (February 08 – July 11, 2019)
Aurel Cerveanu-Hogas (July 10, 2019 – August 09, 2019)

Guests

Prof. Dr. Dirk-Jan Reijngoud (UMCG)

Overview 2019

2019 was a year of consolidation during which many first-year PhD students established themselves in their respective projects. Hjalmar Permentier continued to lead the Interfaculty Mass Spectrometry Center (IMSC) and established it as an integral part of the UMCG/RUG core facility network. This opened the opportunity to make renewed investments in the IMSC to keep the infrastructure up to date and at the forefront of bioanalytical science. Specialized technical personnel is currently the limiting factor to accommodate and run the large number of projects in the IMSC and data analysis is becoming a bottleneck. We hope that this can be alleviated through new positions as part of funded projects and structural investments from the UMCG/RUG.

Peter Horvatovich took more responsibilities in the Department of Analytical Biochemistry (AB) and initiated many collaborations to give this Department a perspective over the longer run. Peter established himself as an internationally recognized scientist in the field of computational mass spectrometry and data analysis and will play an important role in the national infrastructure program X-omics. His collaboration with Lund University and other research groups across the globe is generating increasing output and international visibility.

The year 2019 was quite successful in terms of output, especially peer-reviewed publications. It is noteworthy that the work of Peter Horvatovich contributed considerably to the list of publications (and citations). One example of such a collaboration resulted in a research paper in Analytical Chemistry on the development of a new pre-processing approach for large mass spectrometry imaging data without loss of information delivering high quality ion images. The joint work in the Swedish Cancer Moonshot project resulted in several high ranking publications presenting proteogenomics results for a large cohort of tumors from melanoma patients. One study demonstrated a more stable signal of the BRAF V600E mutation at the protein level compared to detection at the DNA or transcript level highlighting the importance of multi-omics and digital pathology data integration in cancer research.

There were a number of other collaborative projects, notably in the area of proteogenomics and bioinformatics (Peter Horvatovich and Victor Guryev), the combination of electrochemistry and mass spectrometry (Hjalmar Permentier and Rainer Bischoff) and therapeutic proteins (Rainer Bischoff and Nico van de Merbel). While output from these projects, in terms of publications, is still limited, it is expected that this will change in the year 2020.

As our research lines rely on sophisticated and expensive instrumentation, 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. In early 2019 we acquired a new high-resolution mass spectrometer, which 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. Further investment by the UMCG in 2 high-end LC-MS instruments for proteomics was approved and installation will follow in early 2020. In 2020, we will also purchase a high-performance computational cluster to support proteogenomics data integration and pre-processing of large-scale LC-MS datasets.

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 and biomarkers, 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 2020.

Rainer Bischoff, Peter Horvatovich & Hjalmar Permentier

Research Projects

1. Biomarkers

1.1 Cervical Cancer Biomarkers

The cervical cancer project progressed slowly in 2019 due to lack of funding. A grant application to the Dutch Cancer Society to validate our candidate biomarker Minichromosome Maintenance Complex-3 (MCM-3) protein in a larger cohort of women undergoing screening for cervical cancer received no funding. This currently hampers progress, despite the very promising initial results. We will make new attempts of getting this work funded and hope that the long-term investments in this area will ultimately lead to an assay that can discriminate between women that are at risk of developing cervical cancer from 'false positives' after initial testing for high-risk Human Papilloma Virus (HPV) infections.

1.2 Heart Failure Biomarkers

This project is being continued under the leadership of Peter van der Meer (Department of Cardiology and Thorax Surgery, UMCG). Peter received a grant from the Dutch Heart Association in order to screen for activators of the enzyme 5-oxoprolinase (OPLAH), which was shown to be a critical factor in determining outcome after heart failure. Our involvement in this project is to assure that the recently expressed and purified OPLAH is active and that it is suitable to develop a screening assay for potential lead compounds. To this effect, we adapted the LC-MS/MS method to measure glutamate in cell culture-derived material and showed that the recombinant enzyme is indeed active.

1.3 Building a lipidomics analysis platform

In 2019, 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 2020. The XCMS workflow is also being tested by Xiaodong for bacterial lipid extracts (Lu Wang, Medical Microbiology) to determine its suitability for very divergent lipid profiles.

1.4 COPD Biomarkers

In 2019, we developed a validated LC-MS/MS method to quantify Surfactant Protein D (SPD), another prioritized biomarker candidate that we prioritized based on our initial literature study. Using this method, we found that acute exposure to cigarette smoke leads to a statistically significant increase of serum SPD levels. In conjunction with our previous work on the soluble Receptor of Advanced Glycation Endproducts (sRAGE), which decreased after acute exposure to cigarette smoke, this indicates further that this preanalytical factor must be controlled prior to sampling in view of discovering and/or validating novel biomarkers for Chronic Obstructive Pulmonary Disease (COPD).

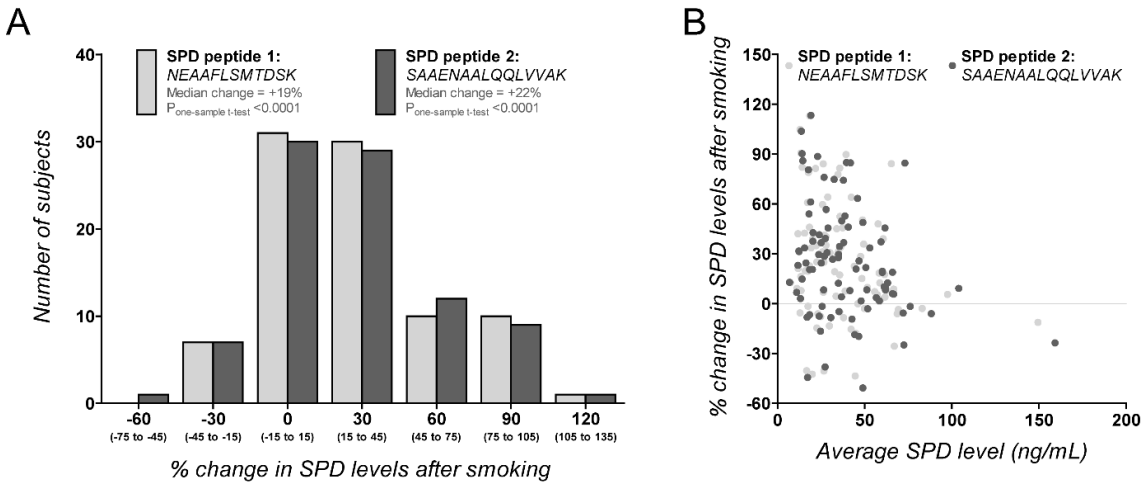

Figure 1: Relative changes between SPD levels measured in serum samples that were taken two hours after smoking three cigarettes within one hour and samples that were taken at baseline (N = 89) presented as (A) histogram and (B) Bland-Altman plot (unpublished data based on our validated LC-MS/MS method and two signature peptides).[1]

1.5 Proteoforms of Biomarkers

In collaboration with the Department of Laboratory Medicine at the University Medical Center Groningen (UMCG), we started a project aiming at the quantitative determination of different isoforms or proteoforms of macromolecular biomarkers. Currently, concentrations of these biomarkers are typically determined using ligand-binding assays such as ELISAs, but there often is a lack of consistency between results obtained at different laboratories, or even within a single laboratory when different lots of critical immunochemical reagents are used. Since most, if not all, protein biomarkers occur *in vivo* as a family of closely related but structurally different isoforms that may respond quite differently in a ligand-binding assay, it is increasingly realized that the generation of a single read-out may be an oversimplification. By using mass-spectrometry based methods, we expect to obtain more knowledge about this important phenomenon. First results indicate that four major isoforms of human growth hormone (hGH) can be separately quantified.

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. 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.

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[1] Klont, F.; Pouwels, S. D.; Bults, P.; van de Merbel, N. C.; ten Hacken, N. H. T.; Horvatovich, P.; Bischoff, R. Quantification of surfactant protein D (SPD) in human serum by liquid chromatography-mass spectrometry (LC-MS). Talanta 2019, 202, 507-513.

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 to provide an open-source version of the Threshold Avoiding Proteomics Pipeline (TAPP), as a major resource for the mass spectrometry community. The current version of TAPP includes 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 advanced GPU programming, such as OpenGL, has been prototyped and will be finalised in 2020. 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 2020/2021. The development of TAPP is supported by the PhD project of Alejandro Sánchez 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 Lund University and Frank Suits, we are further developing a mass spectrometry imaging (MSI) 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, who implemented a new pre-processing algorithm that extracts clean ion images of isotopes of all compounds present in an MSI dataset (see Figure 2). We are further collaborating with the the group of Jeroen Kool and Erika Amstalden (VU, Amsterdam) to acquire protein distribution images in animal tissue with a QTOF instrument and started to establish an MSI platform in collaboration with Prof. Daan Touw (UMCG).

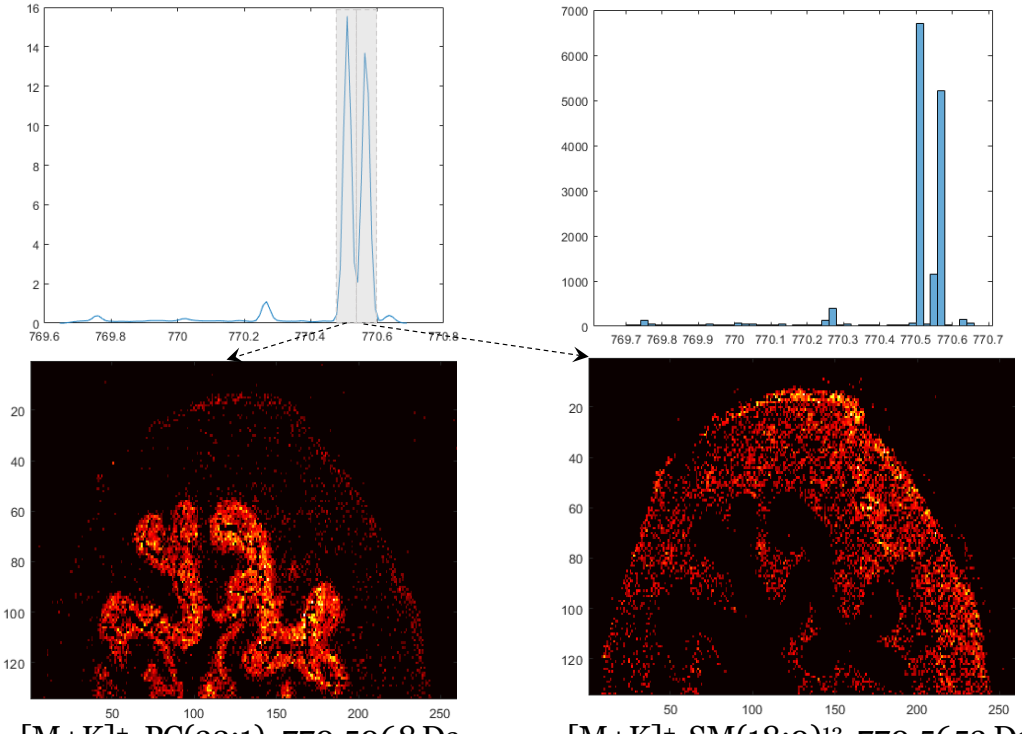

Figure 2: MSI dataset showing ion images of two lipids (phosphatidylcholine, 770.5068 Da and sphingosylphosphorylcholine, 770.5653 Da), who have very similar molecular masses, from a mouse bladder dataset acquired at 10 µm spatial resolution using an Orbitrap mass spectrometer. The data was obtained from ProteomeXchange (PXD001283) and originate from the work of the Spengler group (University of Giessen, Germany).

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 regularly reported on the Wiki edited by Peter (http://c-hpp.web.rug.nl). As of October 2017, Peter Horvatovich is also editing the 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 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 proteins and proteoforms that are related to the pathophysiological, molecular mechanisms underlying COPD. A manuscript from this work based on 8 control and 10 COPD stage IV patients was accepted at the end of 2019 in Thorax (Figure 3) and an extension of this study to analyse 120 human lung tissues from controls and COPD stages I-IV is planned for 2020.

Another proteogenomics project is ongoing in collaboration with György Halmos and Renee Verhoeven (Head and Neck Department, UMCG) which has the aim to reveal 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 tissue was 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 2020.

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

This research line is supported by the X-Omics infrastructure initiative with one PhD position, which is filled by Yanick Hagemeijer starting in February 2020. The goal of this PhD project is to develop an advanced proteogenomics data integration pipeline including accurate prediction of structural variants and the development of a professional workflow that allows simple parametrisation and execution in a high-performance computing environment.

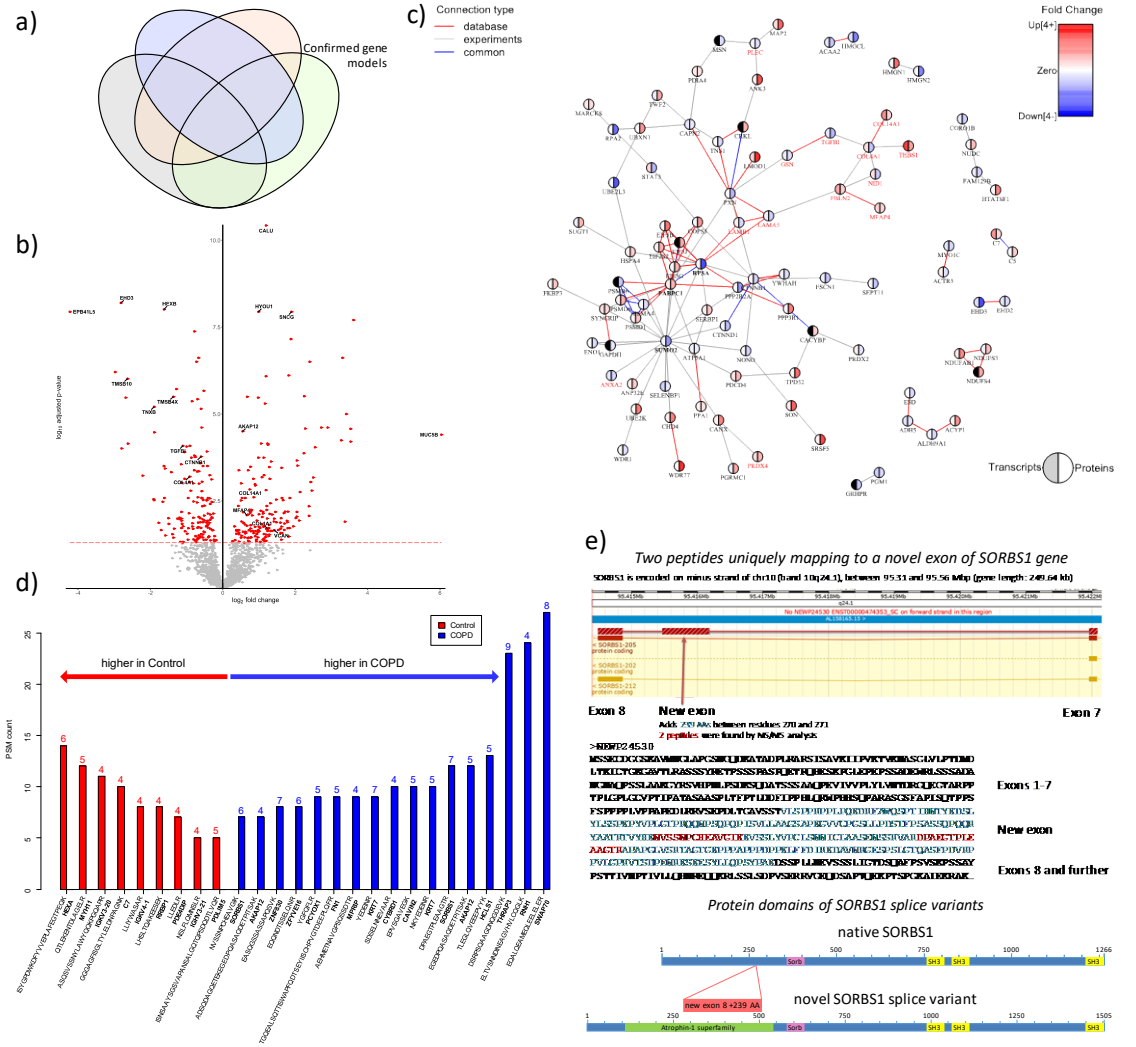

Figure 3: Summary of the COPD proteogenomics data integration paper accepted in Thorax. a) Venn diagram showing the total number of identified peptides that mapped to canonical sequences in the Uniprot and Ensembl public databases (normal text), and non-reference sequences (red bold text), which included non-synonymous variants (single amino acid variants), new transcript isoforms and confirmed gene models. b) Volcano plot of all proteins consistently expressed in COPD and control lung tissue. Differentially expressed proteins (FDR <0.05) are in red. c) STRING protein-protein interaction network based on differential protein expression in severe COPD using an FDR cut-off <0.01. Red connections show known protein-protein interactions from databases, grey connections represent experimentally-derived protein-protein interactions, and blue connections are common database and experimentally-derived interactions. Pie charts express the fold change at the transcript (left) and protein (right) level in severe COPD. The direction and fold change is indicated in blue (downregulated) and red (upregulated). The genes related to the extracellular matrix organization gene ontology are highlighted in red. d) Number of MS/MS spectra (PSMs) attributed to non-reference-sample-specific peptides that were exclusively identified in severe COPD and control lung tissue, respectively. Only peptides with at least 5 PSMs that are present in at least 4 COPD patients or controls were considered. The number of samples where the non-reference peptide was identified is indicated at the top of each bar. e) Upper plot shows the genomic region of the new exon that was identified in the human SORBS1 gene. The arrow indicates the location of an additional exon corresponding to 238 amino acid residues. SORBS1 is encoded on the minus strand of chr10 (band 10q24.1) between 95.31 and 95.56 Mbp (gene length: 249.64 kb). The lower plot shows the amino acid sequence of the new SORBS1 splice variant highlighting the additional novel exon (upper-case light-blue) and the two peptides identified by mass spectrometry (red).

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; now at the Institute of Biochemistry, University of Innsbruck, Austria). 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 and Institute of Biochemistry, University of Innsbruck, Austria).

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, Belqes Ahmad AlJaal) 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 with acute and chronic exposure to identify mycotoxin exposure markers in blood.

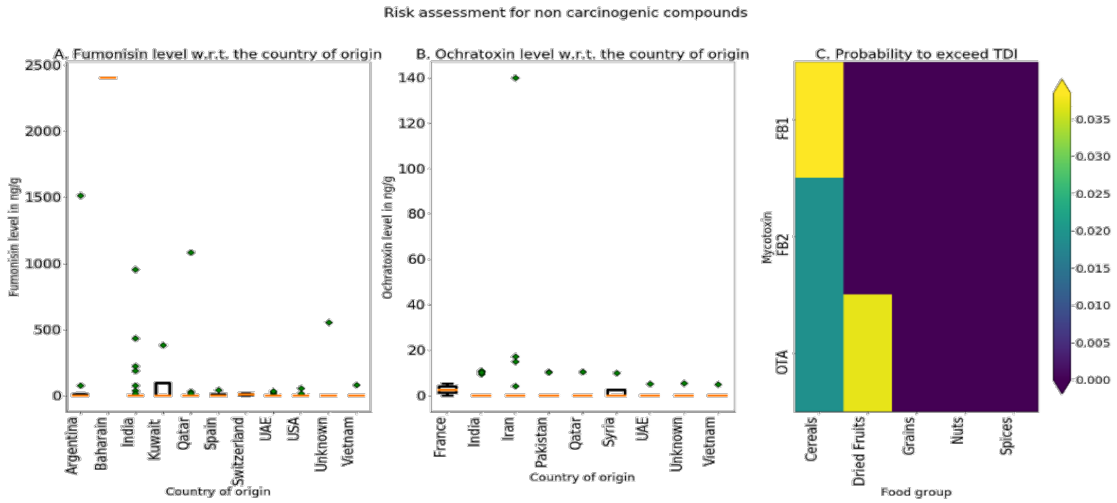

Figure 4: concentration of Fumonisin (A) and Ochratoxin (B) in imported food sold in Qatar according to country of origin. Food risk assessment of Ochratoxin (OTA) and Fumonisin B2 (FB2) and B1 (FB1) according to food categories.[2]

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. This research resulted in 3 accepted

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[2] M Al Jabir, A Barcaru, A Latiff, M Jaganjac, G Ramadan, P Horvatovich, Dietary exposure of the Qatari population to food mycotoxins and reflections on the regulation limits, Toxicology Reports 2019, 6, 975-982

papers in 2019 and we expect more papers from this study in 2020. 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 that 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 (Laboratory Medicine, UMCG). Andrei Barcaru, a postdoctoral scientist from the UMCG, is the 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 analysis of the collected molecular profiles and clinical metadata and to participate in the supervision of a PhD student (Jonatan Eriksson). This project resulted in 4 papers related to melanoma in 2019 and there are additional ones expected in 2020.

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 published in Bioinformatics on correcting the FDR p-value calculation bias of partial correlations and another manuscript published in Scientific Reports on the application of Gaussian Graphical Models for analyzing expression array data of cells from nasal and bronchial epithelial brushes. Plans in 2020 are to finalise the thesis of Victor Bernal and to finish a manuscript on the correction of bias in shrunken partial correlations and the further correction of bias in correlation calculations.

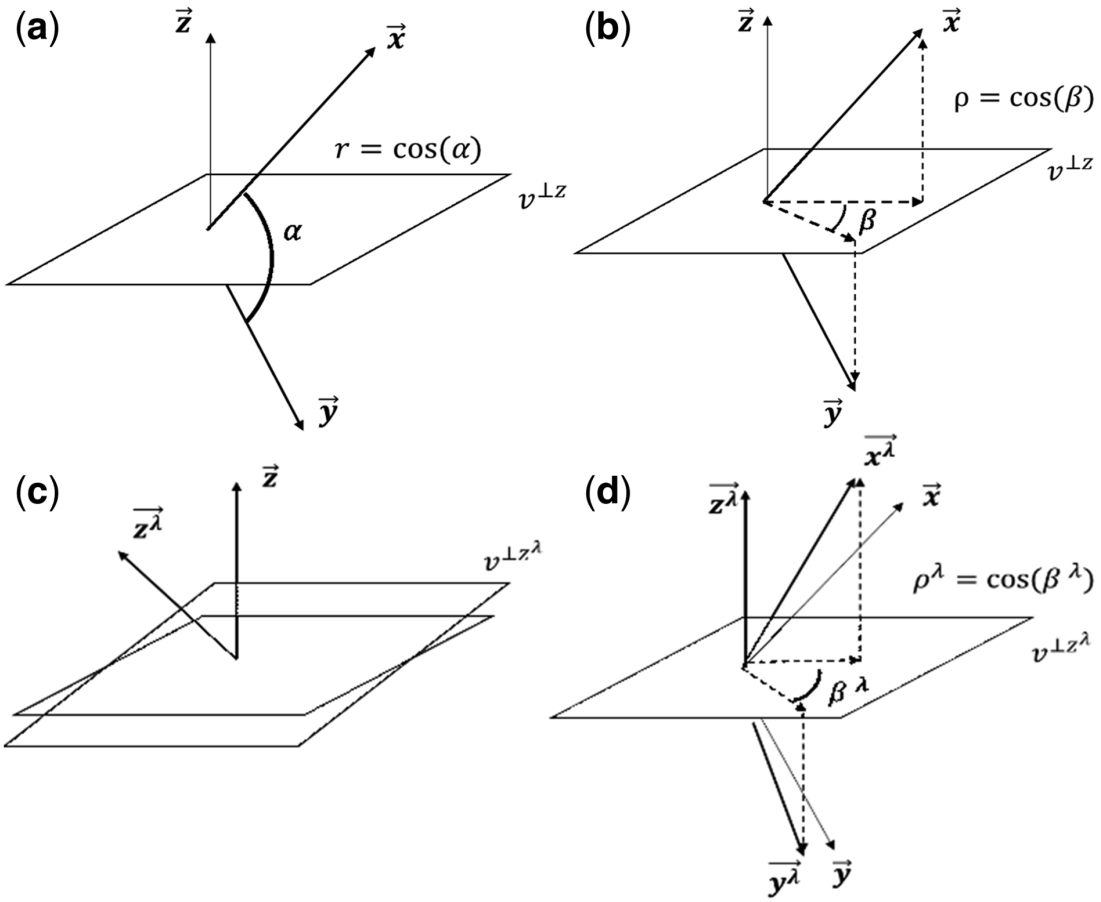

Figure 5: Geometrical representation of partial correlations used in Graphical Gaussian Models. The vectors $\vec{x}$, $\vec{y}$ and $\vec{z}$ represent the random variables *X, Y*, and *Z* in subject space. In panel (a) the correlation $\boldsymbol{r}$ between *X* and *Y* is the cosine of α. In panel (b) the partial correlation between *X* and *Y* can be interpreted as the cosine of the angle β. That is the cosine between the projection of $\vec{x}$ and $\vec{y}$ onto a plane orthogonal to $\vec{z}$. The shrinkage effect consists in that the vectors $\vec{x}$, $\vec{y}$ and $\vec{z}$ are transformed to $\overrightarrow{x^{\lambda}}$, $\overrightarrow{y^{\lambda}}$ and $\overrightarrow{z^{\lambda}}$ such that their lengths remain 1, and only the angles between each other change. In other words, the transformed vectors become less correlated. In panel (c) the geometrical effect of the shrinkage consists in changing the projection planes $\boldsymbol{v}^{\perp \boldsymbol{z}}$ to $\boldsymbol{v}^{\perp \boldsymbol{z}^{\lambda}}$. In panel (d) the "shrunk" partial correlation ρ^{λ} between *X* and *Y* is the cosine of the angle β^{λ}. That is the cosine between the projections of $\overrightarrow{x^{\lambda}}$ and $\overrightarrow{y^{\lambda}}$ onto $\boldsymbol{v}^{\perp \boldsymbol{z}^{\lambda}}$.[3]

3. Drug Targeting, RNA-based Therapy and Chemoproteomics

3.1 Drug targeting and photocleavable mass tags for protein distribution imaging

The research line on Mass Spectrometry Imaging (MSI) 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 MSI 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; now at the Technical University Munich, Germany) and Hjalmar Permentier (Interfaculty Mass Spectrometry Center).

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[3] Bernal, V., Bischoff, R., Guryev, V., Grzegorczyk, M., Horvatovich, P. (2019). Exact hypothesis testing for shrinkage based Gaussian Graphical Models. Bioinformatics (Oxford, England), 35, 5011-5017

In 2019, Jiaying Han published a review on mass spectrometry imaging in Trends in Analytical Chemistry and finished the last chapter of her thesis on the use of a Ru-based metalcomplex-conjugated targeting peptide for laser desorption imaging of protein distributions. Jiaying Han will defend her thesis on February 7, 2020 at the University of Groningen.

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 cellular 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, Diabetes Mellitus 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 funded in 2018 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. 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. The later project line has been extended to include a novel isobaric labelling strategy for peptides.

4.1 Electrochemical peptide bond cleavage and isobaric peptide labelling

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) extended his work to include approaches that go beyond disulphide bond formation as a reversible chemical capture-release approach. He further established a novel chemical approach for the isobaric labelling of peptides based on commercially available reagents. The method is an improvement over the current IPTL approach, where quantitative information is derived from peptide fragment ions instead of reporter ions only. Xiaobo's approach allows for more extensive multiplexing while retaining the benefits of IPTL over the TMT/iTRAQ isobaric labelling methods. We expect to publish on these developments in 2020. 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 4.2 below) are ongoing in close collaboration with Mathieu Odijk and Pascal Führer (PhD student) of the BIOS Group at Twente University.

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), Jos Hermans (research technician) and Adelina Dinter (Erasmus student, University of Münster, Germany) continued investigations of NPG, focussing on various techniques to reproducibly prepare and characterize NPG surfaces by alloy mixing (collaboration with Arne Wittstock, University of Bremen) and electrodeposition or sputtering (collaboration with Twente University). The aim is to prepare flow-through reactors based on electrochemical flow-through cells. The figure below shows that lidocaine can be N-dealkylated on NPG but also that the dealloying conditions are critical. Further work in collaboration with the University of Twente (Mathieu Odijk) are ongoing with defined nanostructured surfaces and the design of a spectroelectrochemical device.

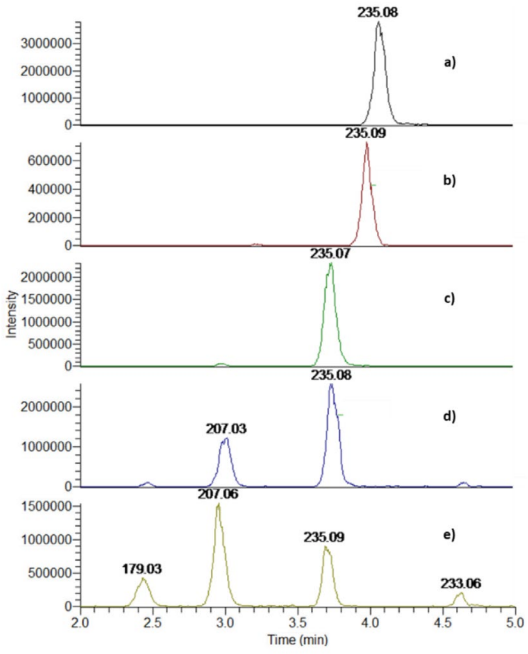

Figure 6: LC-MS chromatograms of lidocaine N-dealkylation reactions on Ag/Au 60/40 w% that was dealloyed in 3 M nitric acid for a) 15 min, b) 1 h, c) 4 h, d) 8 h and e) 16 h. The depicted LC/MS chromatograms show the combined extracted ion chromatograms of m/z: 179, 205, 207 (N-dealkylated product), 233 and 235 (lidocaine) (Alipour, Dinter et al., unpublished).

In parallel to the NPG studies, Ali made significant progress with optimizing parameters for scaling the N-dealkylation reaction of tropane alkaloids and opioids up. Custom-made electrochemical batch reactors now provide gram-scale, high-yield synthesis of noratropine and norscopolamine, which are important precursors for bronchodilator drugs. We are currently in the final stages of the tropane alkaloid work and have encouraging data with the opioids. Part of this work focusses on characterizing some of the minor side products of these reactions by MS and NMR, which will provide further insight in the reaction mechanisms. We anticipate that these reactions will also be studied in more detail in the spectroelectrochemical device in collaboration with Twente University.

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

Julia Aresti works as a PhD student in the Molecular Life and Health (MLH) programme of the RUG on the analysis and function of neuroactive metabolites produced by gut bacteria. This MLH project is a collaboration between the Analytical Biochemistry (Hjalmar Permentier) and the Microbial Physiology groups (Sahar el Aidy).

The production and transformation of neuroactive compounds in the gut by microbiota can have a major effect on the host, leading to changes in neurological disease

states or the effectiveness of neuroactive drugs. The first stage of the project focussed on bacterial transformation of L-DOPA. Samples collected from the cecum of rats were incubated *in vitro* with L-DOPA to determine whether cecal microbiota can metabolize L-DOPA. A new peak was detected in all samples by HPLC-ECD-MS/MS and ultimately identified as hydroxyphenylacetic acid (HPAA). The modified analytical method of combining HPLC-ECD-MS helps to detect and identify gut bacterial metabolites in complex biological samples as well as to quantify them based on both the ECD current and the MS ion signal. We plan to extend the HPLC-ECD-MS/MS method to high-resolution MS to demonstrate its ability to identify additional, less abundant compounds potentially allowing more in-depth metabolomics analyses.

A second study focused on the potential enzymatic conversion of ritalin by certain gut bacteria to ritalinic acid, affecting the response to the drug in ADD patients. *In silico* analysis was used to select a range of gut bacteria with appropriate enzyme homologues for ritalin hydrolysis. Several selected bacteria appeared to be actively producing ritalinic acid, but more detailed study revealed that ritalin is itself highly instable at pH values of 7 or higher. A clear bacterial cause for ritalin break-down in the gut could therefore not be proven, but the pH (in)stability of ritalin deserves closer attention. Further studies into the effect of gut microbiota on other drugs are ongoing.

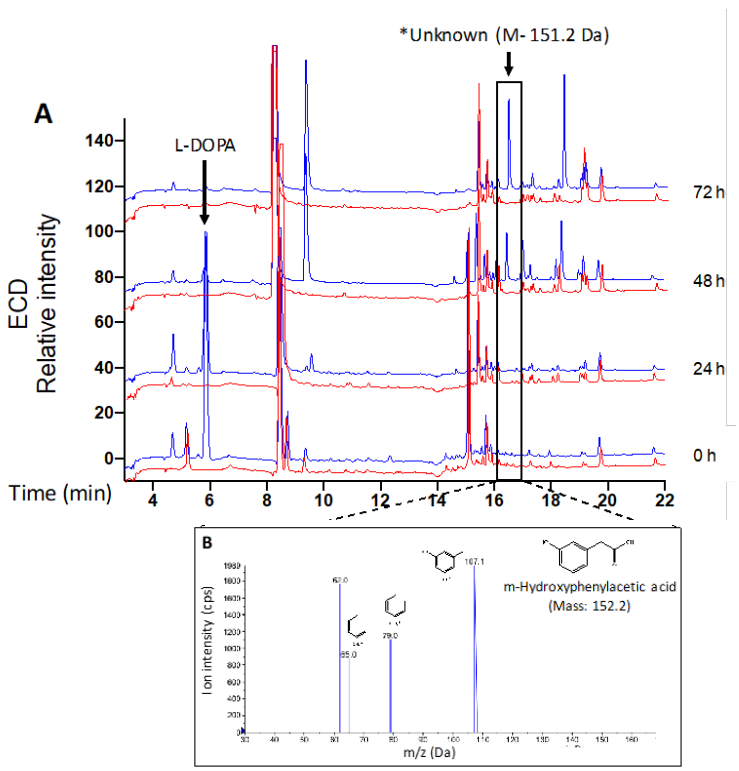

Figure 7: Cecal contents of rats fed L-DOPA (blue) vs control (red). ECD chromatograms (A) reveal a range of expected and unknown metabolites. Metabolites were identified using on-line LC-ECD-MS/MS (B).

5. Proteomics by targeted mass spectrometry

This research line is driven by Karin Wolters, assisted by Ydwine van der Veen. More and more researchers 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, using the selected reaction monitoring (SRM) approach as the main '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/glycolysis-related proteins (coll. Bakker). We are currently developing additional assays related to ER stress (coll. Jonker).

In a collaborative project with the group of Bart van de Sluis, we applied lipid metabolic targets not only for the detection of protein markers in plasma, but also for quantification of proteins in lipoprotein particles after FPLC fractionation, revealing the distribution of different proteins over the fractions. The example in Figure 8 studying Washc1/Commd1$^{\Delta Hep}$ shows that the total amount of APOA1 does not change, but that the distribution of this protein is shifted, indicating larger HDL particles.

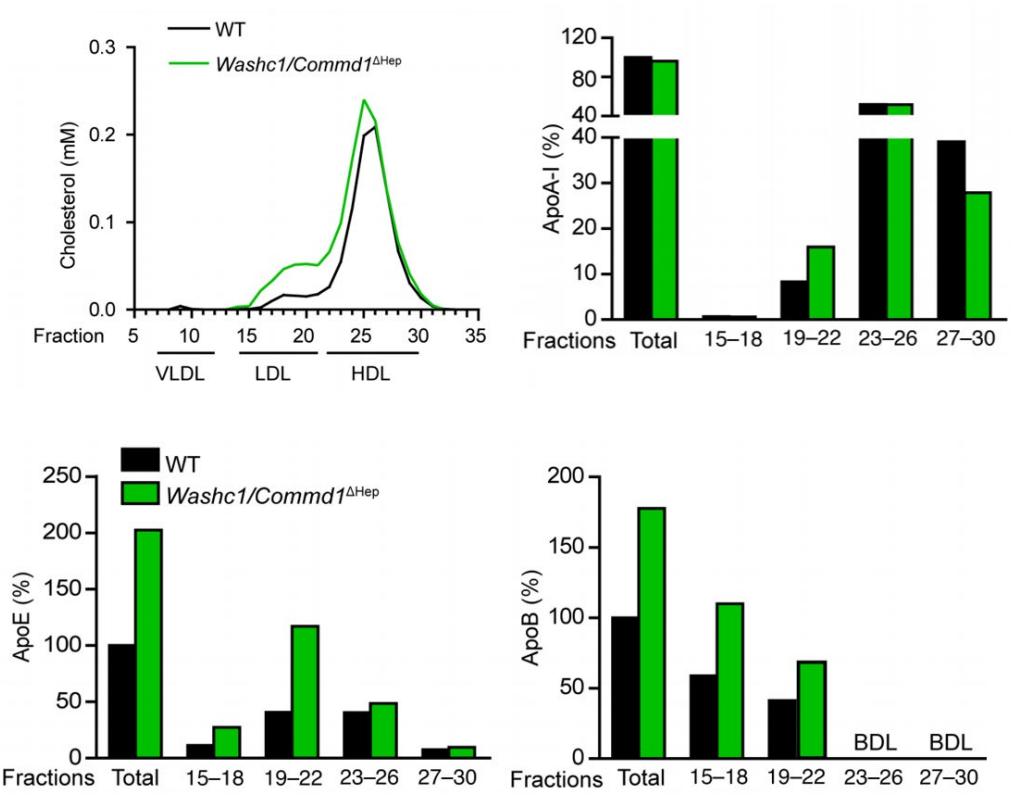

Figure 8: Quantification of protein markers for different lipoparticles. Proteins were quantified from plasma (Total) and from FPLC fractions collected from a run as shown in the upper left panel.[4]

6. Biopharmaceuticals

Our work on the biotransformation of Trastuzumab and Pertuzumab as part of the EU-funded A4B project has progressed along two lines. First, we succeeded in developing a high-performance separation method for proteoforms of both antibodies by chromatofocussing following a secondment of Baubek Spanov at the laboratory of Alois Jungbauer (Laboratory of Protein Technology and Downstream Processing, Austrian Center of Biotechnology, Vienna, Austria). Second, we received a panel of Affimers from Avacta Lifesciences (Wetherby, UK) that are currently under study for the enrichment of Trastuzumab and Pertuzumab from plasma with initial promising results. Our goal is to enrich proteoforms of both therapeutic proteins from patient plasma to follow their *in vivo* biotransformation. To this effect, we entered into a collaboration with the Dutch Cancer Institute (NKI, Amsterdam) to obtain plasma samples from breast cancer patients undergoing combination therapy with both antibodies. In addition, we plan to

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[4] Wijers, M. et al., The hepatic WASH complex is required for efficient plasma LDL and HDL cholesterol clearance. JCI Insight 2019, 4. pii: 126462.

investigate the binding sites of the Affimers using H/D exchange mass spectrometry in collaboration with Hexal/Novartis in Oberhaching (Germany) and ion mobility mass spectrometry in collaboration with the group of Michael Glocker (University of Rostock, Germany).

The research line focusing on the quantitation of biopharmaceuticals at the protein level by LC coupled to high-resolution mass spectrometry (LC-HRMS) as an alternative to the more frequently used approach to quantify them at the peptide level after digestion, has been continued under supervision of Nico van de Merbel. In collaboration with the pharmaceutical company Ferring (Copenhagen, Denmark), we developed an LC-HRMS (Q-TOF mass analyser) approach to quantify recombinant human growth hormone (rhGH, somatropin) in plasma without digestion at the 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.

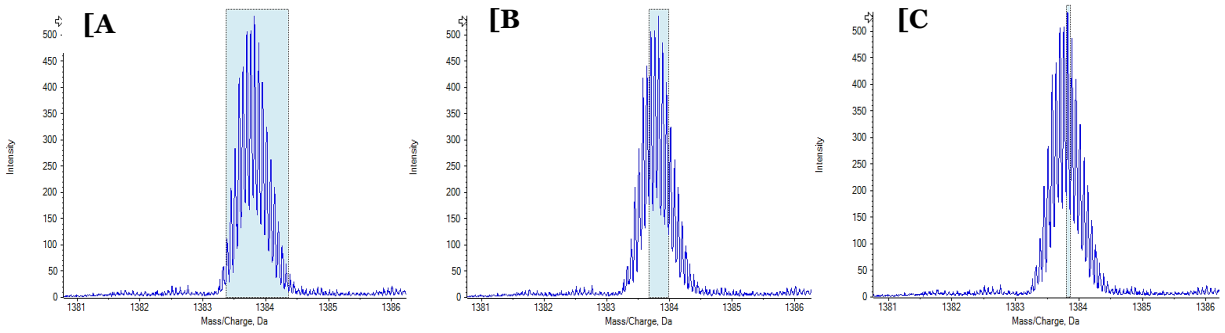

Figure 9: Isotopologues distribution of the 16^+ charge state of rhGH (somatropin) after high-resolution mass spectrometric analysis. Reducing the mass extraction window from 1.0 Da (A) to 0.25 Da (B) and to 0.0625 Da (C) serves to generate extracted ion chromatograms with reduced chemical background noise (Bults et al., submitted).

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

This research line is a collaboration with Marcel Kwiatkowski and Kathrin TheDieck (University of Innsbruck, Austria) and the PhD students Alienke van Pijkeren (2+2 PhD student; currently in Innsbruck) and Sara Russo (PROMINENT PhD student) in close collaboration with Barbro Melgert and Frank Dekker (both GRIP).

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 (e.g. RAW 264.7 macrophages) and different treatments to induce pro-inflammatory responses (e.g. LPS, high fatty acid and glucose concentration). With this cellular model, we study the anti-inflammatory effect of different lysine deacetylase (KDAC) inhibitors (see Figure 10). We analyse pro-inflammatory and anti-inflammatory responses based on changing gene expression and cytokine secretion profiles as well as on macrophage polarization measured by qPCR, ELISA and flow cytometry. To investigate how macrophage polarization and inflammatory gene expression are regulated by changes in energy metabolism leading to changes in the proteome/acetylome, we apply and develop different stable-isotope labelling (fluxomics) technologies targeting histone acetylation dynamics and the dynamics of energy metabolites.

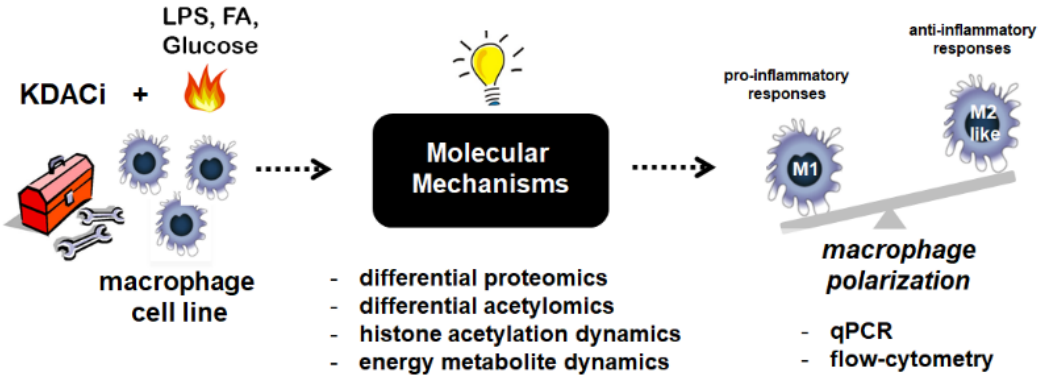

Figure 10: Scheme of the research concept. LPS: lipopolysaccharide, FA: fatty acids

7.1. Site-specific histone acetylation dynamics

In her PhD project, Alienke van Pijkeren develops a methodology to quantify site-specific histone acetylation/deacetylation dynamics. The method is based on a combination of metabolic and chemical stable isotope labeling in cell culture. After establishing a quantitative method for the chemical and metabolic labeling of lysine and acetyllysine residues with stable isotopes and developing a pipeline for bioinformatic data analysis, Alienke introduced tandem MS method that alloes to differentiate between acetylated histone species that are isobaric at the MS1 level, thereby achieving true site-specific acetylation dynamics. Alienke is currently using this method to investigate the spectrum of activity of various KDAC inhibitors to alter site-specific histone acetylation dynamics.

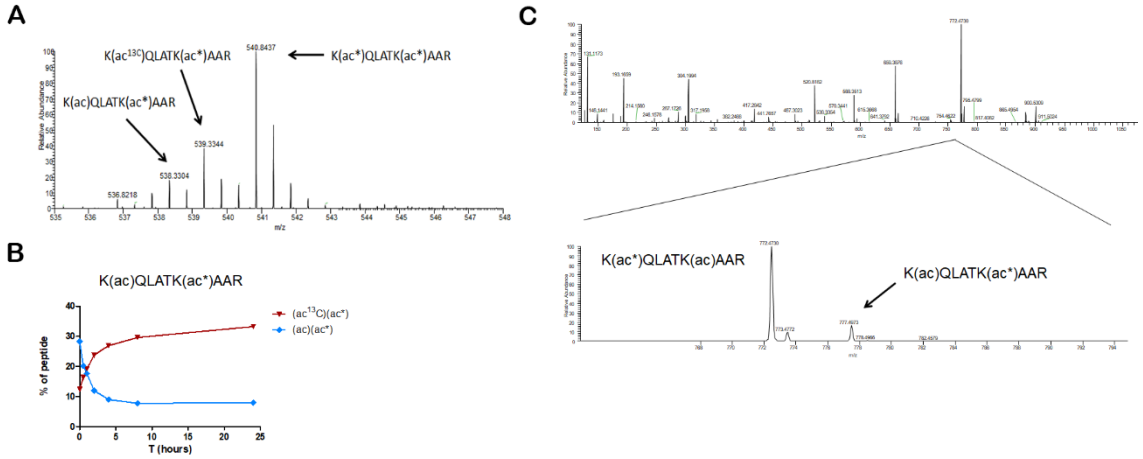

Figure 11: Incorporation of newly synthesized acetyllysine residues into different histone species (panels A and B) and site-specific quantification at the MS2 level (C). ac: endogenous acetyllysine, ac^{13}C: acetyllysine with an acetyl-group obtained from a ^{13}C-labeled tracer molecule (here $^{13}C_6$ glucose), ac*: acetyllysine derived by chemical labeling of lysine residues using $^{13}C_4D_6$ acetic anhydride (unpublished data).

7.2. Regulation of macrophage polarization and inflammation in type-2 diabetes (T2D) and obesity

The second project line within this area focuses on the “regulation of macrophage polarization and inflammation in T2D and obesity through changes in energy metabolism and protein acetylation”. Sara Russo (PhD student) 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 concentrations of palmitate (in complex with BSA) and/or glucose in the presence or absence of anti-inflammatory KDAC inhibitors. Inflammatory macrophage responses and macrophage polarization are investigated by qPCR, flow cytometry and ELISA. In the future, Sara will apply differential proteomics/acetylomics, flux analysis of energy metabolites and our method for site-specific histone acetylation dynamic analysis to investigate how macrophage polarization and inflammation is regulated by changes in energy metabolism and protein acetylation.

8. Interfaculty Mass Spectrometry Center (IMSC)

The IMSC underwent several important changes in 2019. With the official retirement of our very experienced technician Margot Jeronimus in January, Walid Maho has become fully in charge for the LC-MS analyses of small molecules. Walid started as technician in the IMSC in September 2018 and has rapidly established himself in the facility. Together with Marcel de Vries, they handle the proteomics and metabolomics analysis requests, respectively, as well as numerous other smaller projects. Targeted proteomics analysis, as a service, is provided as well, coordinated by Karin Wolters and supported by Ydwine van der Veen.

The range of operational LC-MS instruments was substantially upgraded in 2019 by investment from the IMSC reserves. We were fortunate to be able to acquire two quantitative triple quadrupole LC-MS/MS systems (TSQ Quantum ultra) due to site closure of a pharmaceutical company. These instruments are very useful to cover the high-throughput, medium sensitivity quantitative analyses of small molecules. The ageing LTQ-Orbitrap has been upgraded to an Orbitrap Velos Pro to cover the high-resolution analysis of small molecules, large peptides and proteins, as well as untargeted metabolomics.

The IMSC has also received a very substantial investment by the UMCG in the context of upgrading and updating the UMCG research facilities. In early 2020 two new, state-of-the-art LC-MS systems will be installed, a fast and very high-resolution Orbitrap Exploris 480 and a fast and sensitive TSQ Altis triple quadrupole. These instruments will be primarily used for untargeted and targeted proteomics applications, respectively.

As part of the national X-omics programme we have acquired a TripleTOF 6600+ high-resolution Q-TOF instrument, which is very suitable for data-independent analysis of proteomics samples (e.g. in SWATH mode). To support sample preparation for the expected large cohort studies on the TripleTOF, acquisition of an automated liquid handling and sample preparation instrument is planned in early 2020.

The number of projects and analyses performed at the IMSC for customers has remained at the same level as in previous years (some 40^{+} projects), with a roughly equal division between RUG and UMCG projects. In addition, several projects for companies and other universities were performed, covering both proteomics and small molecule quantitation.

For 2020 we aim for consolidation of the level of service provided by the core staff and the expansion of applications through collaborations with other research departments for, among others, data-independent proteomics, untargeted metabolomics and lipidomics. Expansion of and further investment in data analysis and storage as well as in project management is another point of interest. Leveraging the combined expertise and infrastructure of IMSC, Analytical Biochemistry, and Computational Mass Spectrometry as well as our outside collaborators to supply unrestricted access to high-level mass spectrometry within RUG and UMCG remains the primary goal of our facility.

Ph.D. projects

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

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 (defense scheduled for February 07, 2020)

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 the synthesis of drug metabolites and precursors for pharmaceuticals
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 data-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 functions
Promotor: Sahar el Aidy
Start: April 2018

Bas Sleumer (PRAHS)
Quantification of biomarker isoforms
Promotor: Nico van de Merbel
Start: March 2019

Thesis

Klont, F., Mass spectrometry-based methods for protein biomarker quantification. On the road to clinical implementation. Promotores: Prof.Dr. R. Bischoff, Prof.Dr. P.L. Horvatovich; Co-promotor: Dr. N.H.T. ten Hacken. Dissertation University of Groningen, February 8, 2019, 170 pp (distinction cum laude).

Scientific Output

Scientific publications (peer-reviewed)

1 Al-Jaal, B.A., Jaganjac, M., Barcaru, A., Horvatovich, P., Latiff, A. (2019). Aflatoxin, fumonisin, ochratoxin, zearalenone and deoxynivalenol biomarkers in human biological fluids: A systematic literature review, 2001-2018. Food and Chemical Toxicology, 129, 211-228.

2 Bernal, V., Bischoff, R., Guryev, V., Grzegorczyk, M., Horvatovich, P. (2019). Exact hypothesis testing for shrinkage based Gaussian Graphical Models. Bioinformatics, 35, 5011-5017.

3 Betancourt, L. H., Pawłowski, K., Eriksson, J., Szasz, A.M., Mitra, S., Pla, I., Welinder, C., Ekedahl, H., Broberg, P., Appelqvist, R., Yakovleva, M., Sugihara, Y., Miharada, K.,Ingvar, C., Lundgren, L., Badetorp., B., Olsson, H., Rezeli, M.,Wieslander, E., Horvatovich, P., Malm, J., Jönsson, G., Marko-Varga, G. (2019). Improved survival prognostication of node-positive malignant melanoma patients utilizing shotgun proteomics guided by histopathological characterization and genomic data. Scientific Reports, 9, [5154].

4 Bults, P., Spanov, B., Olaleye, O., Merbel, N.C. van de, Bischoff, R. (2019). Intact protein bioanalysis by liquid chromatography - high-resolution mass spectrometry. Journal of Chromatography B, 1110-1111, 155-167.

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7 Eriksson, J.O., Rezeli, M., Hefner, M., Marko-Varga, G., Horvatovich, P. (2019). Clusterwise peak detection and filtering based on spatial distribution to efficiently mine mass spectrometry imaging data. Analytical Chemistry, 91, 11888-11896.

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9 Han, J., Permentier, H., Bischoff, R., Groothuis, G., Casini, A., Horvatovich, P. (2019). Imaging of protein distribution in tissues using mass spectrometry: An interdisciplinary challenge. TrAC - Trends in Analytical Chemistry, 112, 13-28.

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11 Jabir, M.A., Barcaru, A., Latiff, A., Jaganjac, M., Ramadan, G., Horvatovich, P. (2019). Dietary exposure of the Qatari population to food mycotoxins and reflections on the regulation limits. Toxicology Reports, 6, 975-982.

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15 Lommen, A., Elaradi, A., Vonaparti, A., Blokland, M., Nielen, M.W., Saad, K.A.,, Abushreeda, W.M., Horvatovich, P., Al-Muraikhi, A.E., Al-Maadheed, M., Georgakopoulos, C. (2019). Ultra-fast retroactive processing of liquid-chromatography high-resolution full-scan orbitrap mass spectrometry data in anti-doping screening of human urine. Rapid Communications in Mass Spectrometry, 33, 1578-1588.

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18 Merbel N.C. van de, Koster R.A., Ohnmacht C. (2019). Very complex internal standard response variation in LC-MS/MS bioanalysis: root cause analysis and impact assessment. Bioanalysis 11, 1693-1700.

19 Pijkeren, A. van, Bischoff, R., Kwiatkowski, M. (2019). Mass spectrometric analysis of PTM dynamics using stable isotope labeled metabolic precursors in cell culture. Analyst,144, 6812-6833.

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22 Saad, K., Vonaparti, A., Athanasiadou, I., Saleh, A., Abushareeda, W., Alwahaibi, A., AjabKhan, B.F., Aguilera, R., Kraiem, S., Horvatovich, P.L., Al-Muraikhi ,A.E., Al-Maheed, M., Georgakopoulos, C. (2019). Population reference ranges of urinary endogenous sulfate steroids concentrations and ratios as complement to the steroid profile in sports antidoping. Steroids, 108477

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Book chapters

1. Gil, A., Zhang, W., Wolters, J.C., Permentier, H., Horvatovich, P., Heiner-Fokkema, M.R., Reijngoud, D.-J., Bischoff, R. (2019). Omics | Lipidomics and Its Pitfalls During the Pre-Analytical Stage. Worsfold, P., Poole, C., Townshend, A., Miró, M., (Eds.), Encyclopedia of Analytical Science, (3rd ed.). vol. 8, pp 70–81, Elsevier.

2. Güzel, C., Stingl, C., Klont, F., Tans, R., Willems, E., Bischoff, R., van Gool, A., Luider, T.M. and the Biomarker Development Center Consortium. Targeted proteomics for absolute quantification of protein biomarkers in serum and tissues. In Handbook of Biomarkers and Precision Medicine, (eds. Claudio Carini, Mark Fidock, Alain van Gool), CRC Press. 2019, 408-415.

3. Horvatovich, P., Brandsma, C-A., Suits, F., Bischoff, R., Guryev, V. Proteogenomics and multi-omics data integration for personalized medicine. In Handbook of Biomarkers and Precision Medicine, (eds. Claudio Carini, Mark Fidock, Alain van Gool), CRC Press. 2019, 422-432.

4. Klont, F., Horvatovich, P., Govorukhina, N., Bischoff, R. (2019). Pre- and post-analytical factors in biomarker discovery. In V. Brun , & Y. Couté (Eds.), Proteomics for Biomarker Discovery (pp. 1-22). (Methods in Molecular Biology; Vol. 1959). Humana Press.

5. Merbel, N.C. van de (2019). Sample preparation for LC-MS bioanalysis of proteins, in: Sample preparation in LC-MS bioanalysis, First Edition, W. Li, W. Jian and Y. Fu (Eds.), John Wiley and Sons, pp 304-318.

6. Wolters, J. C., Permentier, H., Bakker, B., Bischoff, R. (2019). Targeted proteomics to study mitochondrial biology. In Targeted proteomics to study mitochondrial biology (pp. 101-117). (Advances in Experimental Medicine and Biology; Vol. 1158). Springer Singapore.

Lectures

1. Klont, F., Assessment of sample preparation bias in mass spectrometry-based proteomics, 18th Annual Meeting of the Netherlands Proteomics Platform, Utrecht, January 11, 2019.
2. Bischoff, R., Biomarker discovery and validation – from shotgun proteomics to targeted methods, Institute of Chemistry, Ljubljana, Slovenia, April 11, 2019

3. Horvatovich, P., 2019, Drug Localization in Oncology, Applying Clusterwise Peak Detection and Filtering Based on Spatial Distribution, Ourcon VII, Saint-Malo, France, October 28-31, 2019
4. Horvatovich, P., Application of proteogenomics data integration to study the effect of genome variability in head and neck cancer and late stage COPD, invited keynote speaker, Italian and Hellenic Proteomics Society meeting, International XIV Congress 2019, Catanzaro, Italy, June 25-27, 2019.
5. Horvatovich, P., Computational mass spectrometry to explore the limits of metabolomics and proteomics profiling, UMCG Technology Symposia, Groningen, December 12, 2019.
6. Horvatovich, P., Exploring the limits of mass spectrometry based molecular profiling, Swedish Cancer Moonshot, invited lecture, Lund, Sweden, October 21, 2019.
7. Horvatovich, P., Exploring the limits of high-resolution mass spectrometry imaging data, 18th HUPO 2019, Adelaide, Australia, September 15-18, 2019.
8. Horvatovich, P., Exploring the limits of mass spectrometry for quantitative molecular profiling, Humbold Colloquium, Madrid, Spain, April 11-13, 2019.
9. Horvatovich, P., Accurate processing of multidimensional liquid chromatography (LC^n-MS/MS) data, DGSM Workshop, Rostock, March 10-13, 2019.
10. Horvatovich, P., The role of proteogenomics to reveal the molecular mechanisms of COPD and Head and Neck cancer, 21th C-HPP workshop, Saint-Malo, France, May 13-14, 2019.
11. Permentier, H.P. Electrochemical oxidation of proteins as an analytical tool. MASSTRPLAN Final Conference, invited lecture, Ghent, Belgium, March 14, 2019.
12. Permentier, H.P. Electrochemistry-mass spectrometry as a versatile tool for drug and protein modification and analysis. PIL lecture, Groningen, October 8, 2019.
13. Bischoff, R., Biomarker discovery and validation – from shotgun proteomics to targeted methods, SMAP2019, Strasbourg, France, September 17-19, 2019.
14. Bischoff, R., Biomarker discovery and validation – from shotgun proteomics to targeted methods, Dutch Clinical Chemistry Society, Utrecht, June 19, 2019.
15. Bischoff, R., Biomarker Best Practices: confounding factors, preanalytics and method validation, 2nd Conference on Validation of Biomarkers, Basel, Switzerland, March 28-29, 2019.
16. Bischoff, R., Bioanalysis of biopharmaceuticals and biomarkers by Liquid Chromatography – Mass Spectrometry, Analytical Technologies Europe 2019, Dublin, Ireland, March 14-15, 2019.
17. Bischoff, R., Bioanalysis of biomarkers and biopharmaceuticals by Liquid Chromatography – Mass Spectrometry, 14th Annual Biomarkers Congress, Manchester, United Kingdom, February 21-22, 2019.
18. Bischoff, R., Biomarker discovery and validation – from shotgun proteomics to targeted methods, FABIAN, Amsterdam, October 18, 2019.
19. Bischoff, R., Biomarkers for Cervical Cancer - the context of use, UMCG Technology Symposia, Groningen, December 12, 2019.

Poster presentations

1. Jonatan Eriksson, Melinda Rezeli, Max Hefner, György Marko-Varga, Peter Horvatovich. Find more compounds in your mass spectrometry imaging data by utilizing localized kernel density estimates of peak masses, 18th Swedish Proteomics Society Meeting, Gothenburg, Sweden, November 24-25, 2019.
2. Jonatan Eriksson, Melinda Rezeli, Max Hefner, György Marko-Varga, Peter Horvatovich. Find more compounds in your mass spectrometry imaging data by utilizing localized kernel density estimates of peak masses, Ourcon VII, Saint-Malo, France, October 28-31, 2019.
3. Horvatovich, P.L. Exploring the limits of mass spectrometry for quantitative molecular profiling, Humbold Colloquium, Madrid, Spain, April 11-13, 2019.
4. Aresti, J., Permentier, H., El Aidy, S. Gut bacteria interferes with levels of methylphenidate; the main treatment in Attention Deficit Hyperactivity Disorder. Annual GBB Symposium, Groningen, August 30, 2019
5. Aresti, J., van Kessel, S.P., Permentier, H., El Aidy, S. LC-ECD-MS for characterization of novel gut bacterial metabolites. 4th NVMS-BMSS conference, Kerkrade, April 1-2, 2019
6. Pouwels, S.D., Klont, F., Kwiatkowski, M.D., Charbonnier, J., Rikxoort, E. van, Bowler, R.P., Bischoff, R. Hacken, N.H.T. ten (2019). Serum levels of the COPD-biomarker sRAGE strongly correlate with specific phenotypes and the progression of emphysema. American Thoracic Society 2019 International Conference, May 17-22, 2019, Dallas, TX, USA
7. Xiaobo Tian, Tao Zhang, Hjalmar Permentier, Rainer Bischoff. Enrichment of electrochemically cleaved peptides for middle-down proteomics applications. ElCheMS 2019-5th International Workshop on Electrochemistry/Mass Spectrometry. Münster, Germany. June 11-12, 2019.

Other presentations

Permentier, H.P.; organization of the UMCG Technology Symposium on Mass Spectrometry, Groningen, December 12, 2019

Editorships/board memberships

Horvatovich, P., Board: Dutch Proteomics Platform

Horvatovich, P., Secretary general, author of HUPOST and PI of Chromosome 5 for Chromosome Centric Human Proteome Project

Horvatovich, P., Member of HUPO and German and Dutch Mass Spectrometry Societies

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)

Research Grants:

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

Biomarker Day Honors College Utrecht students	January 24, 2019
Biotechnology (WLB07045)	February 04 – April 11, 2019
Quantitative Bioanalysis (WMFA14005)	February 04 – 22, 2019
FaTEM (WLFB1210)	February 04 – March 08, 2019
Genomics and Proteomics (WLB07041)	March 18 – April 05, 2019
Advanced Analysis of Biomolecules (Hanzehogeschool, guest lecture)	June 05, 2019
Master d'Analyse des Médicaments, "Introduction à la protéomique" (in French), University of Strasbourg	March 22, 2019
Bachelor thesis & project (WLFB0812 & WLFB0811)	April 15 - June 21, 2019
Medical Genomics & Proteomics (WLB07090)	May 08 – May 28, 2019
Biostatistics (WLFB1001) IIb in 2018/2019 and Ib in 2019/2020 academic year	June 03 - 25, 2019 November 13, 2019 – January 21, 2020
Pharmaceutical Analysis C (WBFA16007)	September 02 - October 11, 2019
Instrumental Analysis	September 02 – 23, 2019
Drug Development (masters)	September 06, 2019
MPDI TOP master (MPDI Topclass 2)	December 06. 2019
Academic Research & Communication Skills (WPFA18001)	Multiple dates between Jan. – June 2019 & between Sept. - Nov. 06, 2019
BMS Proteomics and Proteogenomics data integration	September 30, 2019
Molecular and Cellular Neuroscience (MLBCNN07)	November 21, 2019
Mass Spectrometry (open course)	December 03 – 04, 2019

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., member of the 'toelating commissie', MPS
Horvatovich, P., member of the 'opleidingscommissie', Pharmacy

Special teaching activities

Bischoff, R., September 23 – 27, 2019: EU-COST summer school; Validation of LC-MS/MS methods for the quantification of protein biomarkers: the example of the soluble receptor for advanced glycation endproducts (sRAGE), Spetses Island, Greece.

Merbel, N.C. van de and Trigt, R. van, 23 September 2019: Biomarker course (workshop), 23rd International Reid Bioanalytical Forum, Cambridge, UK.

Student projects

Rien Leuvenink, March - August 2019, Hanzehogeschool, project: Isotope tracing in pro- and anti-inflammatory macrophages - Application of a previously developed metabolite analysis to isotope tracer experiments. Bachelor project supervised by Hjalmar Permentier & Marcel Kwiatkowski

Susan Visscher September 2018 - January 2019, Hogeschool VHL Leeuwarden, project: Isobaric Peptide Termini Labelling for quantification of the proteome. Bachelor project supervised by Xiaobo Tian.

Adelina Dinter, December 2018 – March 2019, University of Münster (Germany), project: Electrocatalytic/catalytic N-dealkylated metabolite synthesis. Erasmus project supervised by Ali Alipour

Andrea Kurtinović, November 2018 – May 2019, IMI international master's, project: Bioanalysis of biotransformation products of Trastuzumab and Pertuzumab. Master's project supervised by Baubek Spanov

Jessica Alferez del Castillo, start November 08, 2019, IMI international master's project: Proteomic profiling in MCAD: towards patient risk assessment. Master's project supervised by Dr. Karin Wolters

Anastasia Audrey, start May 24, 2019, Thermal proteomics-based target landscape of Ibrutinib in childhood B-cell acute lymphoblastic leukemia, external master project at Karolisnka Institute, Stockholm, Sweden supervised by Peter Horvatovich.

Tessa Gillet, (ongoing), master project University of Groningen on "Development and assessment of proteogenomics data integration workflow", supervised by Peter Horvatovich.

Outlook

While continuing along the lines of computational mass spectrometry, biopharmaceuticals, biomarkers and electrochemistry-mass spectrometry, research in the Analytical Biochemistry Department and the Interfaculty Mass Spectrometry Center (IMSC) has also taken new directions. Next to adjustments in the content of our research, there have also been changes related to the research environment. Namely the further integration of Research Core Facilities into a network due to an initiative of the Dean and Vice-Dean of the Medical Faculty. This is a very positive development and will, over the longer run, lead to more concerted efforts to provide cutting-edge enabling technologies to all researchers of the Medical Faculty and the Science and Engineering Faculty at the of the University Medical Center and the University of Groningen. We are looking forward to continuing along this line to ultimately provide an ensemble of research facilities of which the IMSC will be an integral part. This should secure sustained financial support and make the IMSC less dependent on research grants, which will, however, remain a critical part of its funding basis. Hjalmar Permentier (Head of the IMSC) and Karin Wolters (PI, UMCG) are closely involved in these activities.

Another development is the increasing role that Peter Horvatovich plays in defining the research strategy of the Department. Peter has developed in a very successful, independent researcher with a wide network of national and international collaborations. His involvement in the Swedish arm of the worldwide Cancer Moonshot Project (http://www.cancermoonshotlund.com/) has allowed him to develop and apply his extensive knowledge in data processing and biostatistics and resulted in a number of excellent publications. Peter's collaboration with the Center of Excellence in Biological and Medical Mass Spectrometry (CEBMMS) at Lund University (Sweden) on the development of software for the analysis of Mass Spectrometry Imaging (MSI) data has further strengthened this collaboration opening new avenues for research at the Analytical Biochemistry Department in Groningen.

Former postdoc in the group Marcel Kwiatkowski moved to the University of Innsbruck (Austria), where he obtained a permanent position as Head of the Mass Spectrometry Laboratory in the Department of Biochemistry headed by Kathrin TheDieck. Alienke van Pijkeren, joint the group in Innsbruck in October 2019 to continue her PhD work on ''Protein acetylation dynamics – elucidating the connection between energy metabolism and gene expression in age-related inflammatory diseases'' under guidance of Marcel, Kathrin and myself.

The national research infrastructure X-omics (https://www.x-omics.nl/) is taking shape and getting off to a good start under the guidance of Alain van Gool (Radboud University Medical Center, Nijmegen). Peter Horvatovich is the main PI from our side with major involvements in the bioinformatics arm of the infrastructure network. We expect a new PhD student to start working in Groningen in February 2020 to carry the proteogenomics work forward in close collaboration with colleagues of the X-omics network.

Early 2020, we are also looking forward to the installation of new high-resolution and targeted/quantitative LC-MS equipment, liquid handling robotics and an extension of our computational resources. This will enhance our capability of performing cutting-edge 'omics' analyses.

The year ended with a very nice Labday in November organized by Janine, Sara and Walid and our traditional Christmas Dinner in December organized by Karin and Ydwine. The international atmosphere was again touchable and notably 'tasteable'.

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

Peter, Hjalmar and Rainer