university of groningen
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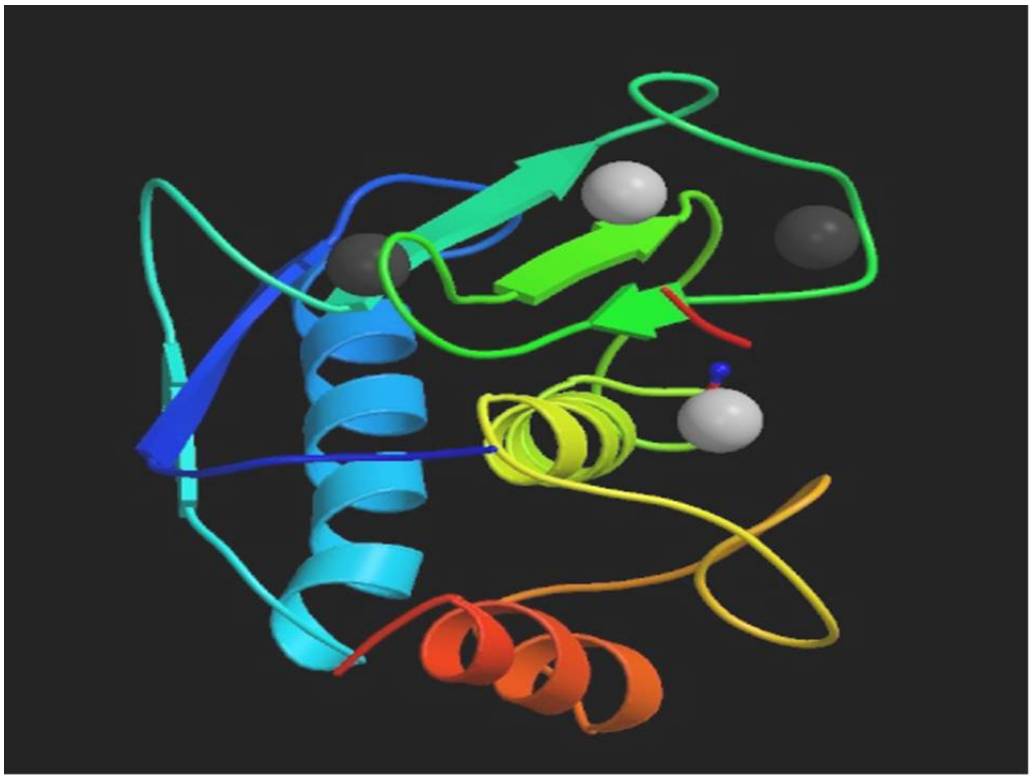
Analytical Biochemistry & Interfaculty MS Center

Annual Report 2020

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

January 28, 2021



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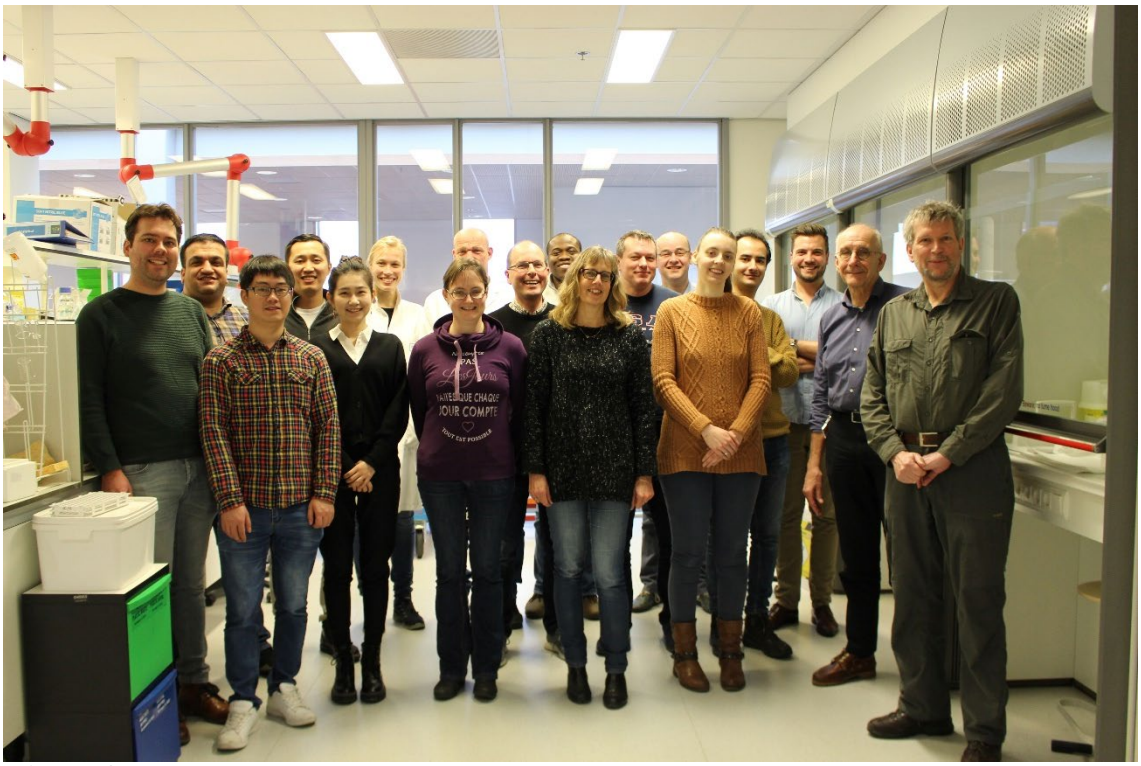

Photo from 2019
From left to right and from back to front:
Bas Sleumer, Walid Maho, Xiaobo Tian, Baubek Spanov, Wenxuan Zhang[1], Ydwine van der Veen, Karin Wolters, Marcel de Vries, Nico van de Merbel, Oladapo Olaleye, Jolanda Meindertsma, Peter Horvatovich, Hjalmar Permentier, Janine Stam, Ali Alipour, Rik Beernink, Dirk-Jan Reijngoud, Rainer Bischoff,
Not on photo:
Jos Hermans, Andrei Barcaru[2], Alejandro Sánchez Brotons, Julia Aresti Sanz, Jan Willem Meints[3], Natalia Govorukhina, Thomas Cremers, Yang Zhang, Alienke van Pijkeren, Saskia Sokoliova, Sara Russo, Victor Bernal Arzola[4], Yanick Paco Hagemeijer

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[1] Left the group in the summer of 2020

[2] Left the group in August 2020

[3] Left the group in September 2020

[4] Left the group in May 2020

Members of the Research Groups

Staff

Prof. Dr. Rainer Bischoff
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 (until September 2020)
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) (until August 2020)

Ph.D. students

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) (thesis defense, September 7, 2020)
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)
Yanick Paco Hagemeijer

Research Students

Victoria Aboagye (start November 09, 2020)
Nazanine Derikvandi
Ikram Yacheur (start February 10, 2020)
Valeriia Ladyhina
Dominique ter Maat (start September 07, 2020)

Guests

Prof. Dr. Dirk-Jan Reijngoud (UMCG)

Personal Message

2020 was a special year for everybody. In February 2020, we thought that the SARS-CoV2 epidemic, which manifested itself in Wuhan (China), was a far-away event that would not affect us very much. This situation changed dramatically in March when the first cases arose in Europe with a spread so fast that health-care services in many countries and regions could not cope with it. The world was in the grip of a pandemic of unprecedented dimensions since the Spanish Flu. This led to the first lockdown, which affected our work significantly. We were no longer allowed to enter the labs and offices for about 2 months except for checking on instruments from time to time. Needless to say that this slowed everyone down in their progress towards a successful thesis or other research-related activities. In addition, we had to move our teaching activities online, a situation that has not changed ever since.

All of this forced us to find new ways of interacting and of getting things done despite these impediments. I would like to compliment everybody in the group, who contributed to this. Ten months later, we are in the second lockdown after a couple of months of limited freedom. The virus is tenacious and does not go for half measures, so we adapted our working style to online work leaving the lab space to those that need it most, the experimentalists. In view of this situation, I must say that I am very impressed with the results that we achieved in 2020. It was not a normal year, far from it, with fewer presentations and conference visits but still with a considerable number of top-level publications and innovative research results that we can all be proud of.

The pandemic showed also that we can pull together and organize the lab according to the rules and regulations handed to us by the Dutch Government via the Security Region Groningen and finally the University and the University Medical Center. In this context, I would like to give special thanks to Karin Wolters and Hjalmar Permentier, who, over the months, have managed lab occupancy and organized lab access. This has worked very well and has become almost second nature to most of us, despite the fact that we would like very much to go back to the 'old days'. While we had a couple of coworkers and students that were in contact with SARS-CoV2-positive people, none has tested positive thus far. Maybe we've just been lucky but I think that our system of diligent occupancy tracking has contributed to this and gives us the feeling that we still have things under control, as much as that is currently possible.

Let us hope that 2021 will be a somewhat more normal year, despite the fact that the current situation does not look rosy. Many hope that large-scale vaccination campaigns will break the spread of the virus, which would be a victory of science and defy the increasing number of people resorting to obscure and often irrational arguments to fight the pandemic. We can all be proud of being part of this community of natural scientists based on whose knowledge and perseverance progress is built and who do not fall for politically motivated arguments that have no bearing on reality. Our group has shown time and again that we can work together across nationalities, religions and ethnic backgrounds and that the worldwide community of natural scientists has common values and a common understanding of the world, which does not go for convenient truths that are not based on facts. I am proud to be part of this community and I am proud to be head of this research group.

2021 will be my last year 'in office' with my retirement planned for the end of the year. It will be a big change in my life, which has been dedicated to research and development for over 40 years in academia and in industry. I wish my successor a good start and all of you success in your future scientific endeavours but most of all I wish you good health. I'll be around as long as I still have PhD students under my responsibility, so you will also see me after 2021 from time to time.

Rainer Bischoff

Research Projects

1. Biomarkers

1.1 Building a lipidomics analysis platform

In 2020, 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. Xiaodong Feng started a new project on the improvement of lipid identification assessing various spectral matching scoring methods for fragment ion spectra and is participating in the assessment of lipidomics and metabolomics LC-MS/MS datasets in collaboration with Hermie Harmsen (bacterial lipidomics) and JJ Schuringa (cancer research). The analytical platform and the XCMS workflow is operational and will be completed with more accurate lipid and metabolite identification in 2021.

1.2 Proteoforms of Biomarkers

In collaboration with the Department of Laboratory Medicine at the University Medical Center Groningen (UMCG) and PRA Health Sciences, we started a project aiming at the quantitative determination of different proteoforms of protein biomarkers. Currently, concentrations of these biomarkers are typically determined using ligand-binding assays such as ELISAs, but there is often 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. Applications of the developed algorithms to clinical translational research, such as biomarker discovery and proteogenomics data integration, lipidomics and antidoping analysis are on top of his research agenda.

Two PhD defenses were held in 2020, that of Jiaying Han on February 7, 2020 and of Wadha Masoud Abushreeda on September 7, 2020. Victor Arzola Bernal submitted his thesis to the Assessment Committee at the end of 2020. Despite the COVID-19

pandemic, the year of 2020 was productive, resulted in 10 peer-reviewed papers from this research line with many others expected to come in 2021.

2.1 Pre-processing LC-MS(/MS) and mass spectrometry imaging data

Alejandro Sanchez Brotons is taking the lead for the development of Pipelines and Systems for Threshold Avoiding Quantification (PASTAQ) to be used in shotgun proteomics as well as in other 'omics-type' LC-MS/MS data sets. The development is based on the Threshold Avoiding Proteomics Pipeline (TAPP) developed in collaboration with Frank Suits (IBM Research, Australia). We aim to make PASTAQ and open source version of TAPP as a major resource for the mass spectrometry community for quantitative pre-processing of LC-MS(/MS) proteomics and metabolomics data. The current version of PASTAQ includes linking the annotation of MS/MS scan information and peptide or metabolite identities to isotopologue peaks in the LC-MS/MS data. The pipeline has been finalized and its performance tested. A publication is at an advanced stage of composition. Efficient and interactive visualisation of large-scale LC(GC)-MS(/MS) data using advanced GPU programming, such as OpenGL, has been prototyped. Finalisation of this platform and adaptation 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 2021/2022.

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. This pipeline comprises a powerful mass spectrum alignment algorithm (MSIWarp) based on correlation optimized warping and the overlap of the Gaussian peak area of mass spectral peaks. We are further collaborating with 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).

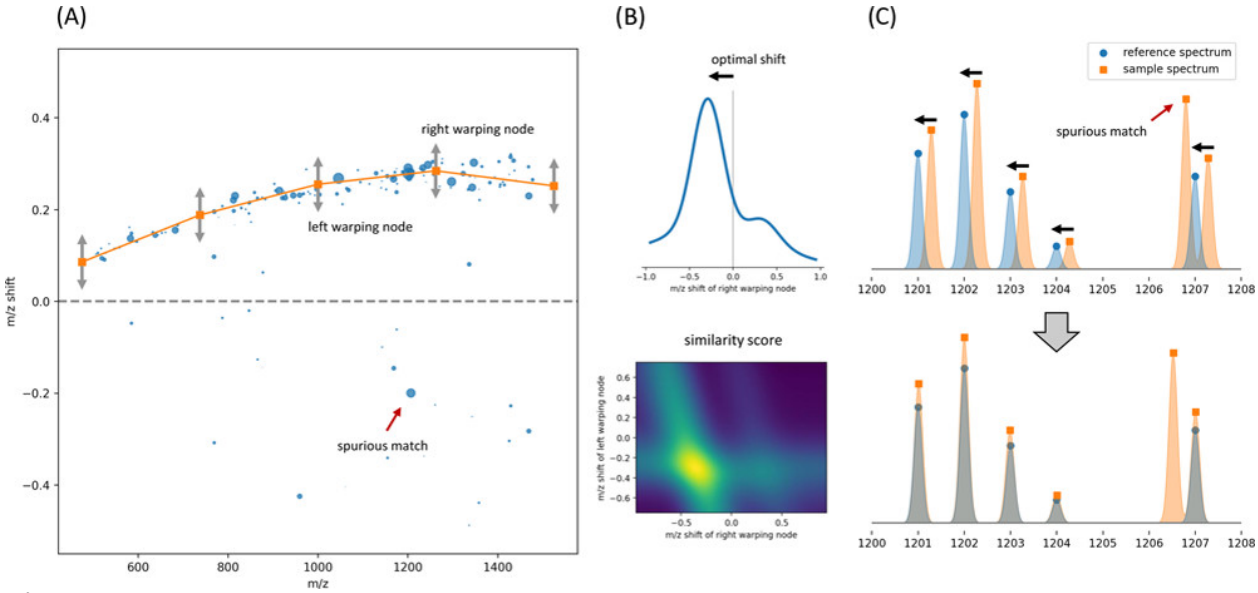

Figure 1: Main step of MSIWarp using the sum of overlapping Gaussian peak areas with correlation optimized warping to align all spectra to a common reference in the MSI data. The paper entitled "MSIWarp: A General Approach to Mass Alignment in Mass Spectrometry Imaging" was published in Analytical Chemistry, 92, 2020, 16138-16148.

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, to report evidence of human protein coding genes at the protein level, the so called missing proteins, and to find at least one function for each protein with evidence at the protein level with a currently unknown function. 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 published in 2020 in Thorax (Figure 2) and an extension of this study to analyse 120 human lung tissues from controls and COPD stages I-IV is currently ongoing.

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. In a 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 has been finished and a paper is currently in the writing phase.

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 filled by Yanick Hagemeijer as of 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. The high throughput proteogenomics data integration pipeline, which is simple to parametrize and run is currently under development by Yanick Hagemeijer.

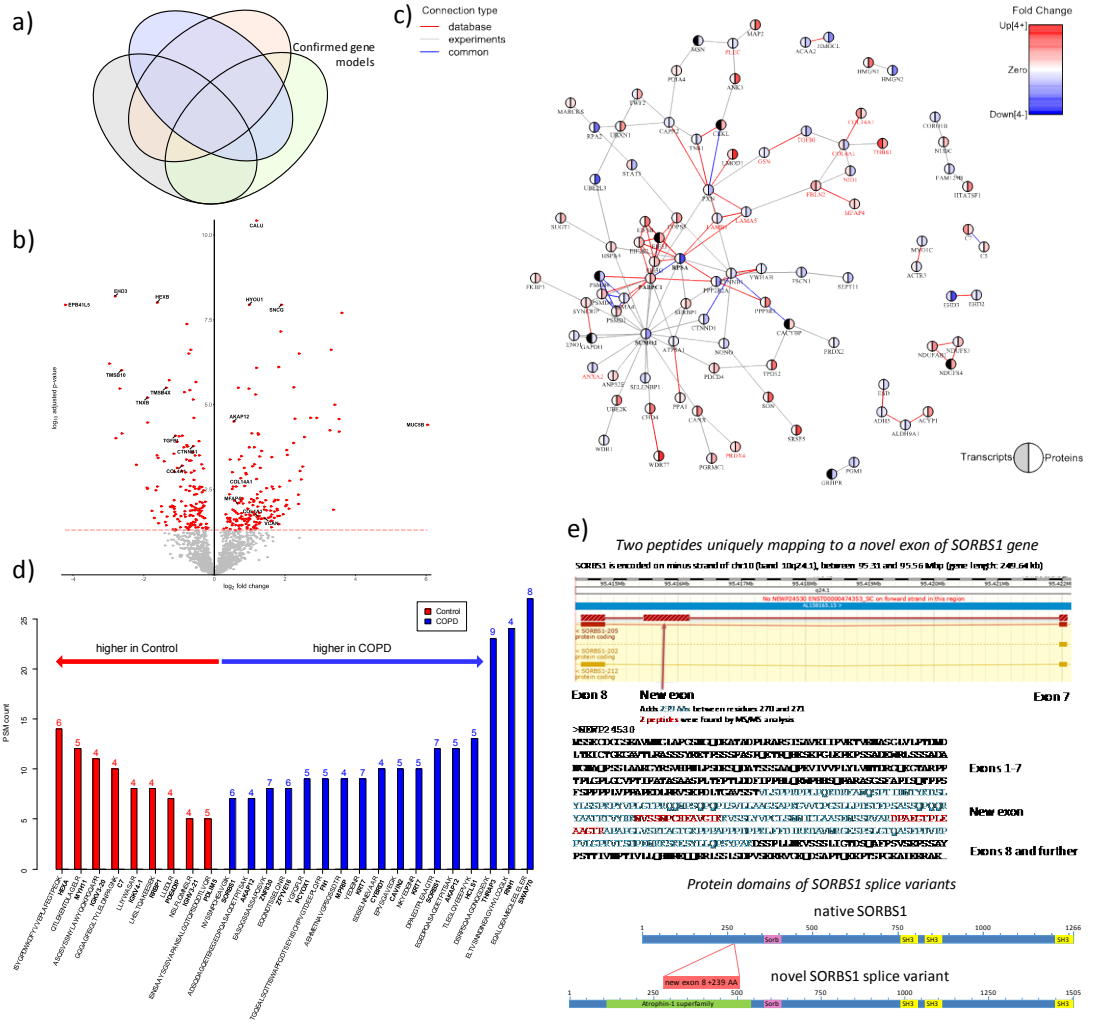

Figure 2: Summary of the COPD proteogenomics data integration paper.[5] a) Venn diagram showing the total number of identified peptides that mapped to canonical sequences in the Uniprot and Ensembl public databases (normal text), and non-reference sequences (red bold text), which included non-synonymous 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).

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[5] Brandsma, C.-A.; Guryev, V.; Timens, W.; Ciconelle, A.; Postma, D. S.; Bischoff, R.; Johansson, M.; Ovchinnikova, E. S.; Malm, J.; Marko-Varga, G.; Fehniger, T. E.; van den Berge, M.; Horvatovich, P. Integrated proteogenomic approach identifying a protein signature of COPD and a new splice variant of SORBS1. Thorax 2020, 75, 180-183.

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, Estonia and Austria. In this project we develop a robust quantitative phosphoproteomics pipeline, which enables us to assess protein phosphorylation changes in tumor tissue. Besides, we 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 is co-supervised by Natalia Govorukhina as well as Kathrin Thedieck, Marcel Kwiatkowski and Alexander Heberle (Institute of Biochemistry, University of Innsbruck, Austria). She is currently finishing her PhD with multiple papers to be published in 2021.

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 was performed in collaboration with researchers (Aishah Latif, Thomas Michael Harvey, Morana Jaganjac, Belqes Ahmad AlJaal) of the Anti-Doping Laboratory of Qatar (ADLQ) and was 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. This project ended in 2020 resulting in a number of papers, but still some data from this project need to be analysed with further publications expected in 2021.

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, was the main collaborator in this project until his departure in August 2020.

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 two PhD student, Jonatan Eriksson and Bea Szeitz. This project resulted in a number of publications and we expect additional papers to be published in 2021.

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

Victor Bernal Arzola 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. Another manuscript focussing on removing the shrinkage effect on the partial correlations is expected to be published soon in BMC Bioinformatics. Victor Arzola Bernal has submitted his thesis at the end of 2020 and it is currently reviewed by the Assessment Committee.

2.9 Improved Phosphosite localisation using predicted mass spectra

The collaboration with Liang Qiao at Fudan University on the application of machine learning predicted mass spectra for improvement of phosphosite localisation was a great success, which resulted in a paper in the Journal of Proteome Research. This project had the aim to assess how various machine learning tools and post processing steps, such as the mass shift according to the phosphorylation sites, phosphoric acid neutral loss, and a "budding" strategy help to provide accurately predicted mass spectra of phosphopeptides and how these spectra facilitate localisation of phosphosite.

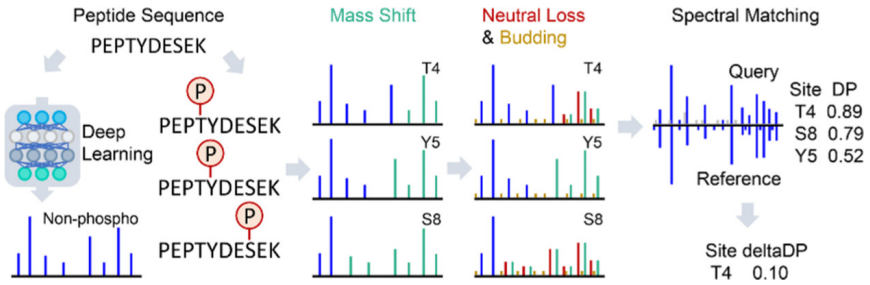

Figure 3: Overview of the Phosphosite Localization Bioinformatic Workflow using fragment ion mass spectra predicted by machine learning. Peptide sequences are the starting input parameter to predict MS/MS spectra using deep learning models trained with the data of non-phosphorylated peptides. Then, the predicted spectra are altered by mass shift according to phosphorylation sites, adjusted by adding phosphoric acid neutral loss fragment peaks and adopting a budding strategy, i.e., adding in a small amount of all possible theoretical fragment ions of the phosphopeptide to the MS/MS spectra. Spectral similarities are computed between the query spectra and the simulated spectra of each candidate site for phosphosite localization. The top match with the highest dot product (DP) score and the deltaDP between the top and the second matches are reported.

The collaboration with the group of Liang Qiao will continue with a joint project supervising the PhD research of Enhui Wu on a proteometagenomics topic.

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 (Technical University Munich, Germany) and Hjalmar Permentier (Interfaculty Mass Spectrometry Center).

In 2020, Jiaying Han published a proof-of-concept paper on the use of a Ru-based metalcomplex-conjugated targeting peptide for laser desorption imaging of protein distributions in Chemical Communications. Jiaying Han has successfully defended her thesis on February 7, 2020 at the University of Groningen.

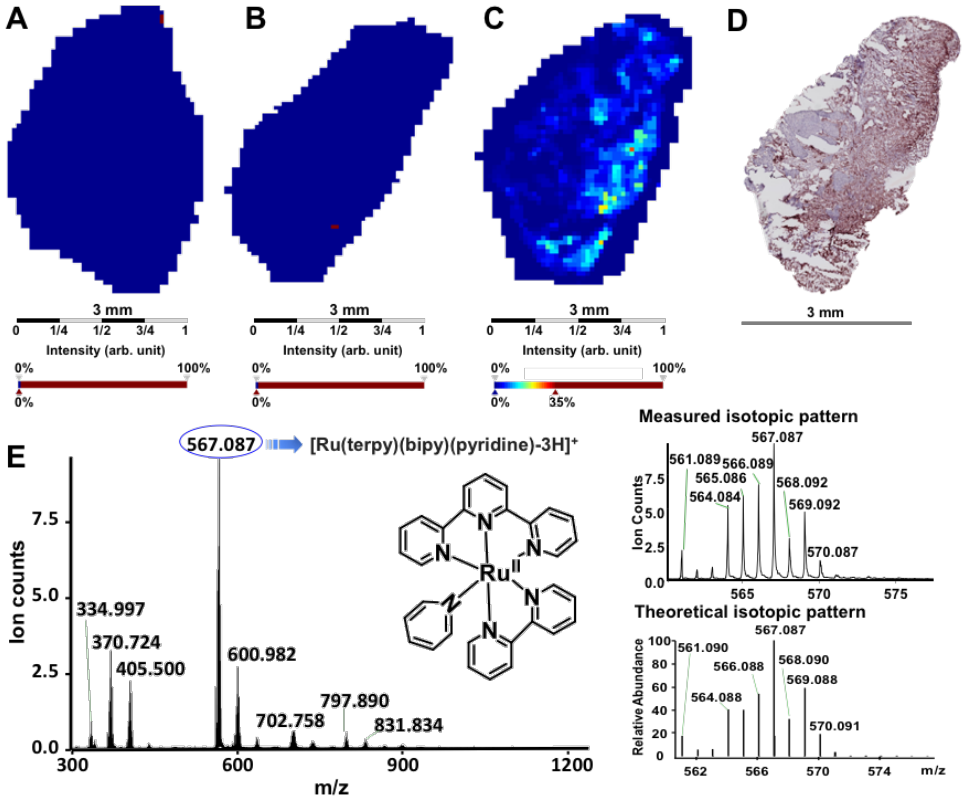

Figure 4: LDI-MSI images of hypopharynx tumor tissue sections incubated with (A) PBS buffer (pH 7.4), (B) compound 2 $[Ru^{II}(terpy)(bpy)(D\text{-biotin})]^{2+}$ and (C) compound 3 $[Ru^{II}(terpy)(bpy)(D\text{-biotin})(cyc(RGDfK))]^{2+}$. Images were obtained with an *m/z* range of 560.43 – 571.91 Da, which covers all isotopes of the Ru mass tags in the LDI spectrum. (D) $\alpha v\beta_3$ IHC and haematoxylin stained section of a hypopharyngeal squamous cell carcinoma section. The dark red staining represents the localization of the target integrin. (E) LDI mass spectrum of the mass-tag signal related to the image of sections incubated with compound 3 (pixel coordinates: x 25, y 34, pixel ID 131). The inset shows the experimental isotopic pattern distribution of the main fragment ion $[Ru(terpy)(bipy)(pyridine)\text{-}3H]^+$ vs the theoretical pattern (mass range from m/z 560 to 575). Images A, B and C were obtained with the "weak denoising" option of the SCiLS software. Figure from Chemical Communications, 2020, 56(44),5941-5944.

3.2 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). Saskia Sokoliova is a PhD student working on this ambitious project with co-supervision by Martin Witte. Saskia Sokoliova synthetized many new reagent for specific AGE products, which we aim to publish on in 2021.

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 electrochemically-assisted synthesis of added value pharmaceutical intermediates.

4.1 Electrochemically-assisted synthesis of value-added pharmaceutical intermediates

A major breakthrough in 2020 was publication of our work on the selective electrochemical N-demethylation of tropane alkaloids to their nortropane derivatives in Green Chemistry based on the work of Ali Alipour (PhD student) in collaboration with Zhangping Xiao from the group of Frank Dekker (GRIP, RUG). Nortropanes, such as noratropine and norscopolamine, are important intermediates for the semi-synthesis of the medicines ipratropium or oxitropium bromide, respectively. Synthesis was performed in a home-made electrochemical batch cell using a porous glassy carbon electrode or a flow-through system. The reaction proceeds at room temperature in one step in a mixture of ethanol or methanol and water. Mechanistic studies showed that the electrochemical N-demethylation proceeds by the formation of an iminium intermediate which is converted by water as the nucleophile. The optimized method was further applied to scopolamine, cocaine, benzatropine, homatropine and tropacocaine, showing that this is a generic way of N-demethylating tropane alkaloids to synthesize valuable precursors for pharmaceutical products.

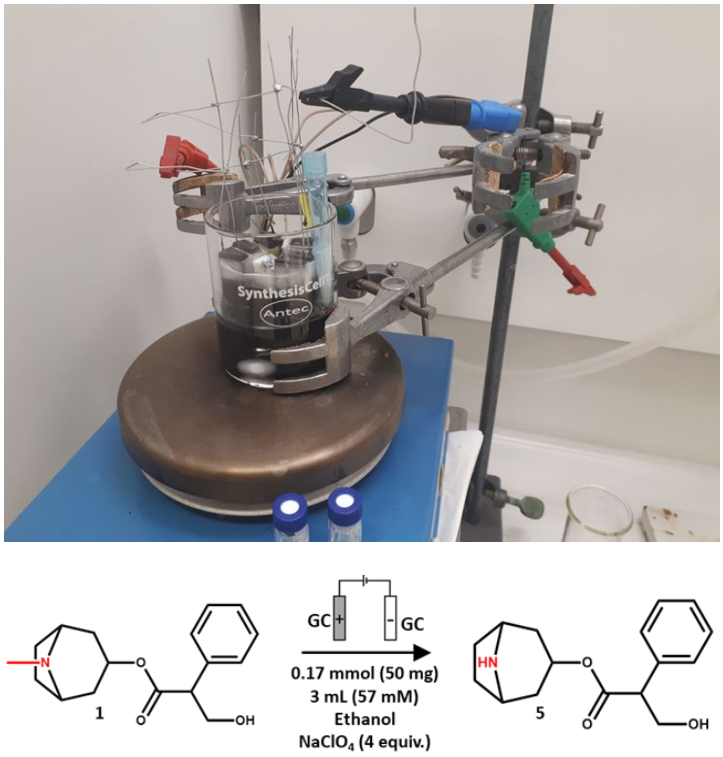

Figure 5: Top panel: Home-made electrochemical cell with a stack of four paired porous graphite electrodes for gram-scale synthesis.
Bottom panel: Reaction scheme and conditions for the N-demethylation of atropine.

Current work focusses on the N-dealkylation of opiates to synthesize important precursors for pharmaceuticals such as naloxone. N-dealkylation of oxycodone to its nor-form proceeds in a similar system as used for tropane conversion, but requires the addition of an electrocatalyst. Gram-scale yields for a range of opiates is possible with both the batch cell and flow cell configuration. The system comprises low-cost, low-energy consumption, environmentally friendly components that can be readily set up in any laboratory.

4.2. N-Dealkylation of pharmaceuticals on gold particles

This project is a 'spin-off' from the original project to synthesize drug metabolites that are due to N-dealkylation mediated by members of the Cytochrome P450 enzyme family. As reported previously, we discovered that such reactions can proceed without any electrical potential on the surface of nanoporous gold. However, obtaining reproducible results has been difficult and the physical-chemical parameters that paly a role in this reaction remained partially unexplored.

To shed more light onto this reaction, Jos Hermans (research technician) and Ali Alipour (PhD student) constructed a capillary column filled with gold particles that was coupled to a mass spectrometer. That way, reaction products could be analyzed 'on the fly' and different reaction conditions were be tested. Jos advanced this project by setting an intricate system up that further allowed to add oxygen and various other reagents that we considered to critical for this reaction. It is noteworthy, that dealkylated lidocaine, our test compound, occurs in good yield already in the flow-through of the gold particle column indicating that the reaction is extremely fast.

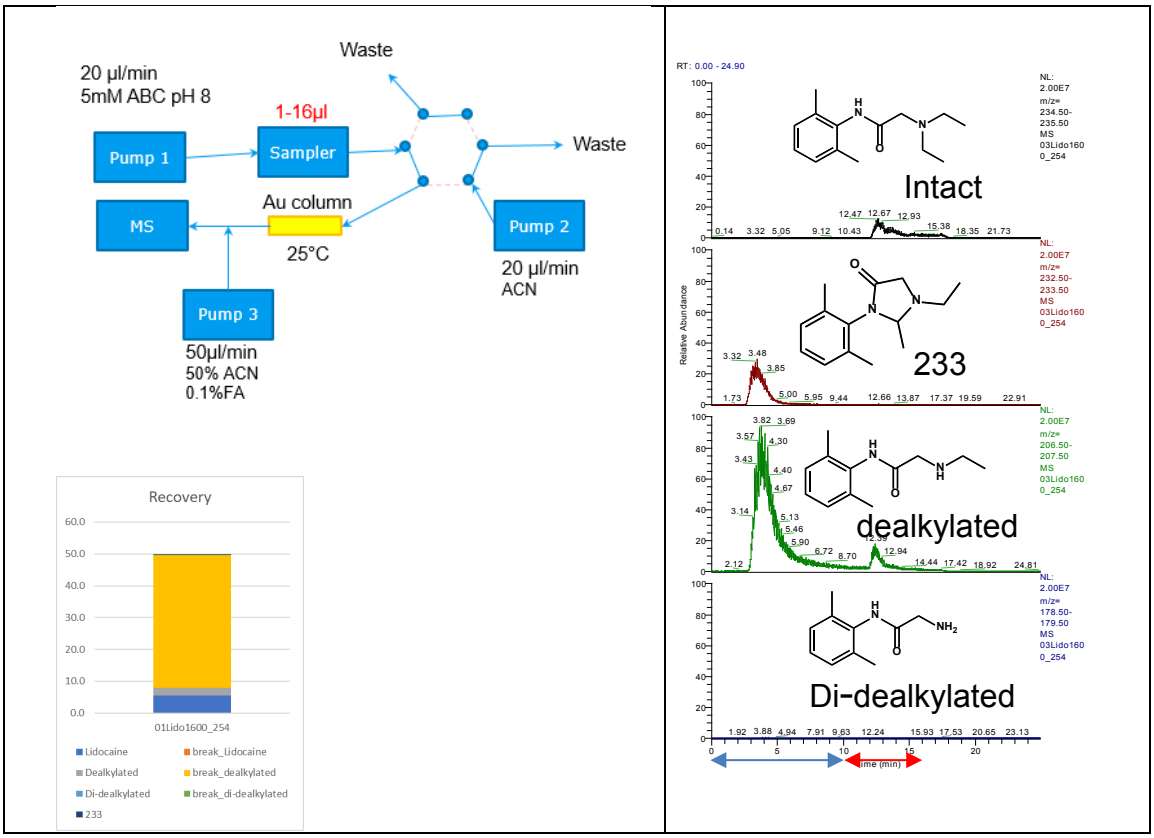

Figure 6: Basic instrumental configuration and chromatographic profiles for a 1µl, 100µM Lidocaine injection. The upper left panel shows the instrumental set-up, while the lower left panel shows a plot of the various compounds that are followed using this set-up. The right panel shows the corresponding mass spectrometric traces as extracted ion chromatograms.

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 programme of the RUG on the analysis and function of neuroactive metabolites produced by gut bacteria. This MLH project is a collaboration between Analytical Biochemistry (Hjalmar Permentier) and the Microbial Physiology group (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. Rat cecal samples were incubated *in vitro* with L-DOPA to determine whether cecal microbiota can metabolize it. A new peak was detected in all samples by HPLC-ECD-MS/MS and ultimately identified as hydroxyphenylacetic acid.

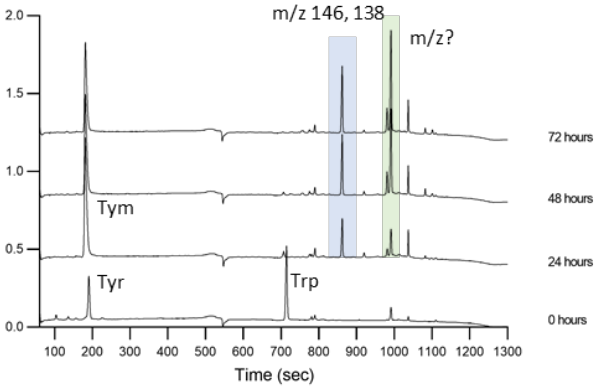

Figure 7: ADD patient fecal sample culture. Metabolite ECD profiles 0-3 days after addition of Tyr and Trp.

The modified analytical method of combining HPLC-ECD-MS helps to detect and identify gut bacterial metabolites in complex biological samples and their quantification based on both the ECD current and the MS ion signal. We have started to implement the HPLC-ECD-MS/MS

method on a high-resolution Q-ToF MS to demonstrate its ability to identify additional, less abundant compounds allowing more in-depth metabolomics analyses. This proof-of-principle study is done on fecal samples of ADD patients which had been dosed with methylphenidate (MPH, ritalin) and cultures of the fecal sample.

Another study focused on the potential enzymatic conversion of MPH by 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 been proven, but the pH stability of ritalin deserves closer attention. Further studies into the effect of gut microbiota on other drugs are ongoing.

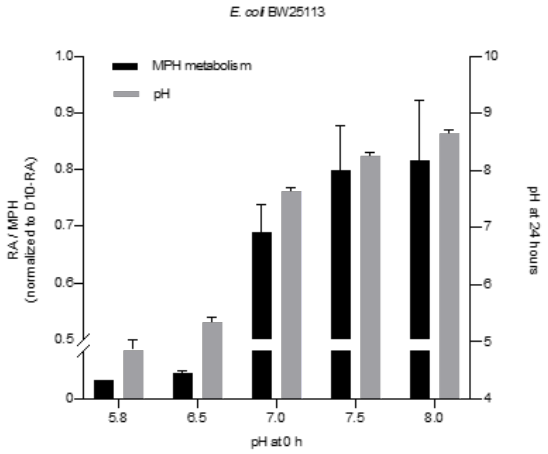

Figure 8: Apparent *E. coli* metabolism of MPH to ritalinic acid as a funtion of pH and the measured pH after 24 h growth.

Finally, the role of the neuroactive peptide substance P and its breakdown in the gut by microbiota was studied. Various bacterial strains have already been shown to be able to digest substance P into specific peptide fragments of substance P. Development of an LC-MS/MS method for its detection in more complex (gut) samples is in progress.

5. Proteomics

5.1 Targeted protein analysis

This research line is driven by Karin Wolters, assisted by Ydwine van der Veen (Dept. of Pediatrics, UMCG). 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'. Internally we are currently applying 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). Last year we also established an Enabling Technology Hotel (https://www.dtls.nl/technology-hotels/list/tools-for-systems-biology-applications/) for targeted proteomics applications, which resulted already in funding for new developments of assays for protein panels in collaboration with Hans Waterham (Amsterdam Medical Center).

In a collaborative project with the group of Maaike Oosterveer (UMCG), we showed the potential of combining multiple panels when analyzing liver samples derived from a mouse model for glycogen storage disease type 1a (GSD 1a), which develops non-alcoholic fatty liver disease. The screening helped in the identification of transmembrane

6 superfamily member 2 (TM6SF2) as a new transcriptional target of carbohydrate response element binding protein (ChREBP). Figure 9 shows TM6SF2 mRNA and protein levels in hepatocyte-specific GSD 1a (L-G6pc$^{-/-}$) mice, as well as the effect of reducing the hepatic expression of the transcription factor ChREBP (using a short hairpin RNA targeting ChREBP, i.e., shChREBP).[6]

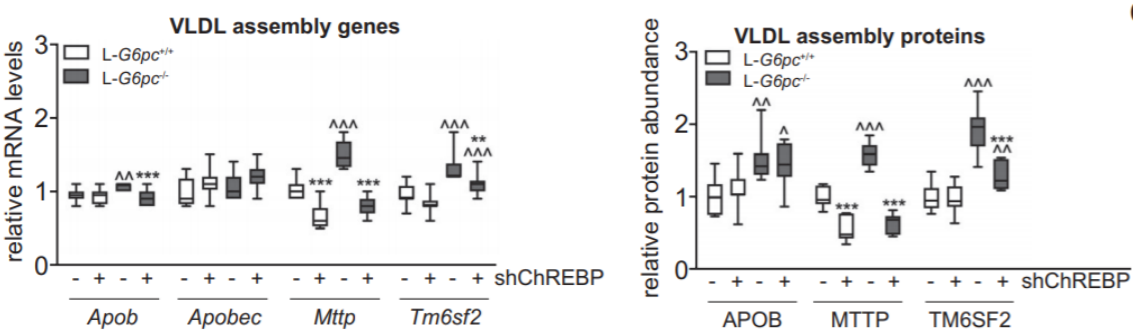

Figure 9. Detection of transmembrane 6 superfamily member 2 on mRNA (left panel) and protein (right panel) level (PMID: 32083759). The L-G6pc$^{-/-}$ mouse model is used as a model for hepatic Glycogen Storage Disease type 1a and hepatic ChREBP knockdown was induced using a short hairpin RNA targeting ChREBP.

5.2 Novel chemical labelling strategies in quantitative proteomics

Advancements in liquid chromatography and mass spectrometry over the last decades have led to a significant development in mass spectrometry-based proteome quantification approaches. A widely used strategy is multiplex isotope labeling, which significantly improves the accuracy, precision and throughput of quantitative proteomics in the data-dependent acquisition (DDA) mode. In 2020, Xiaobo Tian (PhD student) developed a number of novel chemical labelling strategies that can be used in DDA as well as in the more recently developed data-independent acquisition (DIA) mode. These novel chemical tags overcome some of the limitations of reporter-ion-based DDA approaches, such as ratio distortion due to co-fragmentation of more than one precursor ion.

The figures below show examples of tags that are based on different principles. Figure 10 shows an isobaric, peptide fragment-ion-based quantification approach, which conserves the merits of quantifying peptides based on unique fragment ions while reducing the complexity of the b-ion series compared to conventional fragment ion-based quantification methods. Figure 11 schematically shows an alternative approach that is suitable for DDA and DIA in which the quantitative information is concealed in isobarically labeled peptides and revealed upon tandem MS in the form of peptide-coupled reporter ions. Xiaobo is currently developing additional tags that are even more suitable for multiplexed DIA.

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[6] Lei, Y.; Hoogerland, J. A.; Bloks, V. W.; Bos, T.; Bleeker, A.; Wolters, H.; Wolters, J. C.; Hijmans, B. S.; van Dijk, T. H.; Thomas, R.; van Weeghel, M.; Mithieux, G.; Houtkooper, R. H.; de Bruin, A.; Rajas, F.; Kuipers, F.; Oosterveer, M. H. Hepatic Carbohydrate Response Element Binding Protein Activation Limits Nonalcoholic Fatty Liver Disease Development in a Mouse Model for Glycogen Storage Disease Type 1a. Hepatology 2020, 72, 1638-1653.

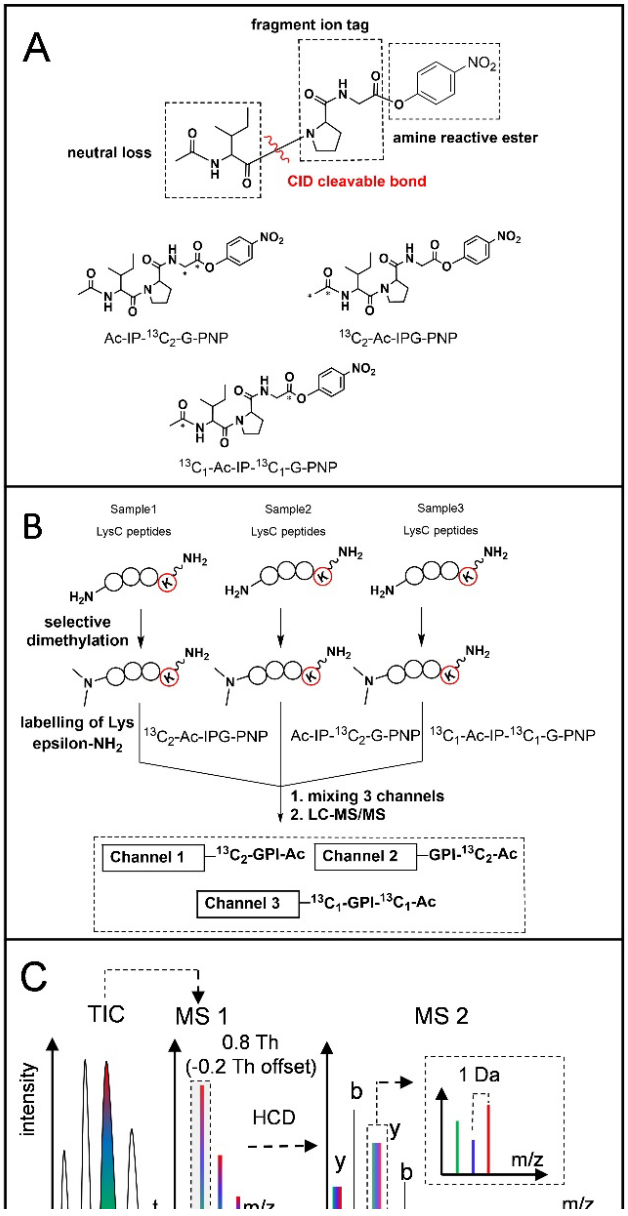

Figure 10: Schematic view of the Ac-IPG approach. A) Functional design of the Ac-IPG-PNP tag (^{13}C isotope locations of the triplex Ac-IPG-PNP tag are marked with "*"); B) triplex isobaric labeling steps; C) LC-MS/MS for a mixture of triplex labeled samples (from: Tian, X.; de Vries, M. P.; Permentier, H. P.; Bischoff, R. A Collision-Induced Dissociation Cleavable Isobaric Tag for Peptide Fragment Ion-Based Quantification in Proteomics. Journal of Proteome Research 2020, 19, 3817–3824).

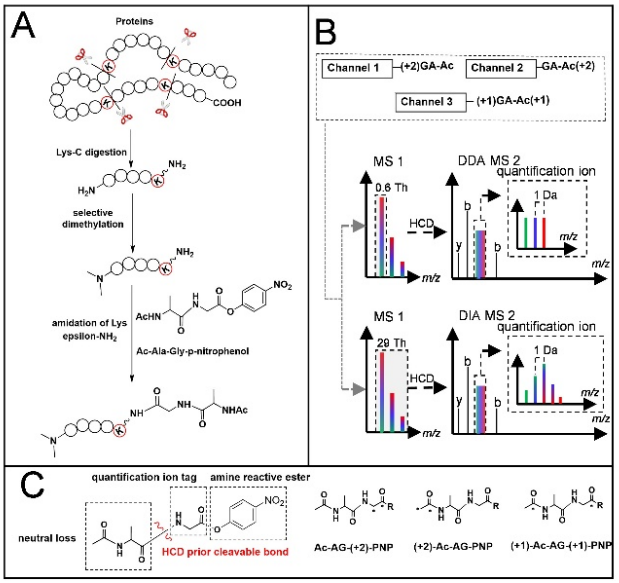

Figure 11: Schematic view of the Ac-AG approach. A) Isobaric labeling steps; B) LC-MS of a mixture of triplex-labeled samples in DDA mode; C) Functional design of the triplex-labeled Ac-AG-PNP tag (^{13}C isotope locations are marked with "*"). From: Tian, X.; de Vries, M. P.; Permentier, H. P.; Bischoff, R. A Versatile Isobaric Tag Enables Proteome Quantification in Data-Dependent and Data-Independent Acquisition Modes. Analytical Chemistry 2020, 92, 16149–16157.

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 we screened for their suitability to enrich Trastuzumab and Pertuzumab selectively from plasma. Our goal is to enrich proteoforms of both therapeutic proteins from patient plasma to follow their *in vivo* biotransformation. To this effect, we recently received a range of plasma samples from patients undergoing Trastuzumab/Pertuzumab combination therapy from a clinical study at the Dutch Cancer Institute (NKI, Amsterdam). We have also started to investigate the binding sites of the Affimers using H/D exchange mass spectrometry in collaboration with Hexal/Novartis in Oberhaching (Germany) and by ion mobility mass spectrometry in collaboration with the group of Michael Glocker (University of Rostock, Germany). Unfortunately, these collaborations have been hampered by restrictions related to the COVID-19 pandemic.

6.1 Developing an enrichment method for therapeutic antibodies

The detailed study of the *in vivo* biotransformation of monoclonal, therapeutic antibodies in breast cancer patients requires efficient means to selectively enrich these target proteins from blood plasma without interference by other IgGs or plasma proteins. To this end we continued our collaboration with Avacta Lifesciences, who provided us with a range of affimers that bind Trastuzumab or Pertuzumab selectively.

Oladapo Olaleye (PhD student) evaluated these affimers using a plate-based enrichment method, which resulted in the prioritization of 4 affimers from each group. These affimers were immobilized on Ni^{2+}-NTA or maleimide beads based on a 6-His tag or a single cysteine that was engineered into the affimers. An example of the screening results is shown below.

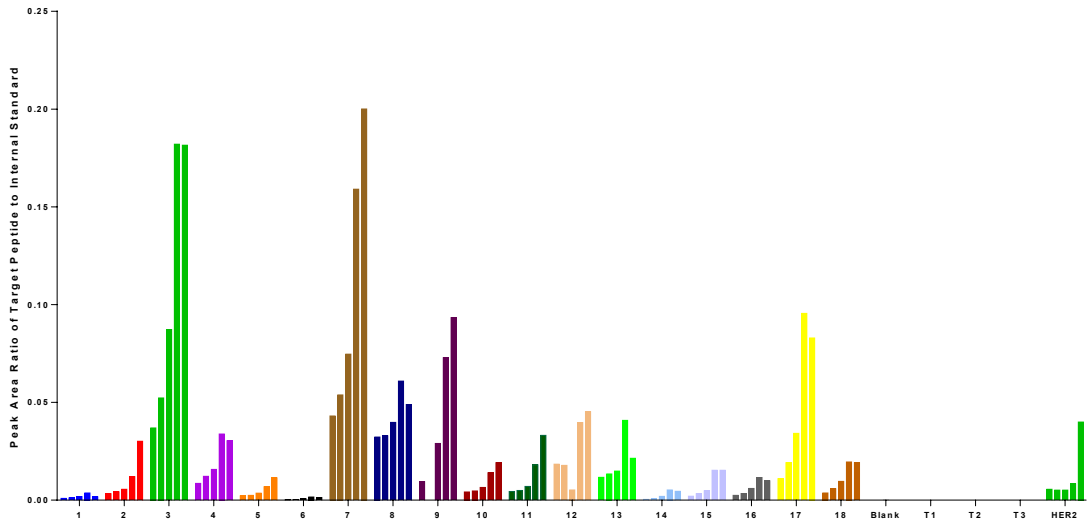

Figure 12: Evaluation of anti-Pertuzumab affimers for enriching the therapeutic antibody from plasma. Affimers 3, 7, 9 and 17 were prioritized for further work. Pertuzumab was added at 75, 150, 300, 750 and 1500ng to 100µL plasma. Affimers T1-T3 are anti-Trastuzumab affimers (negative controls) and Her-2 is the extracellular domain of the Her-2 receptor (positive control).

6.2 Developing a chromatographic separation method to study monoclonal antibody heterogeneity

As therapeutic, monoclonal antibodies are heterogeneous by nature and since this heterogeneity will likely increase further upon *in vivo* biotransformation, it is indispensable to have a very powerful separation method at the protein level to ultimately link modifications to individual proteoforms and possibly to activity. To this end we developed a cation-exchange chromatographic method with a highly controlled, stable pH gradient elution to separate proteoforms from Trastuzumab (and later also from Pertuzumab). This method is based on work by the group of Alois Jungbauer (Laboratory of Protein Technology and Downstream Processing, Austrian Center of Biotechnology, Vienna, Austria). While complete separation of all proteoforms is beyond any chromatographic method at present, we achieved unprecedented resolution, giving rise to some 15 fractions that we are currently characterizing further. Ultimately, we will combine this separation method with the enrichment method described above to study the *in vivo* biotransformation of Trastuzumab and Pertuzumab in breast cancer patients undergoing combination therapy on samples obtained from the Dutch Cancer Institute (NKI) in Amsterdam.

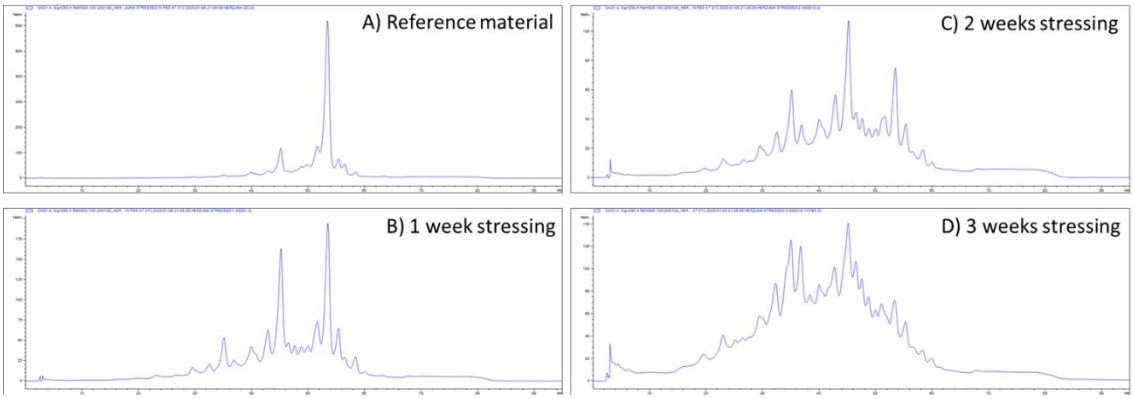

Figure 13: Separation of Trastuzumab proteoforms by cation-exchange HPLC with pH gradient elution showing evolution of the charge-state-related profile upon incubation at 37°C in PBS.

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) performed by the PhD students Alienke van Pijkeren (2+2 PhD student; currently in Innsbruck) and Sara Russo (EU-funded PhD student on the PROMINENT project) in close collaboration with Barbro Melgert (GRIP, RUG).

In this project we aim to identify novel molecular mechanisms that drive macrophage polarization towards a pro-inflammatory M1-type phenotype or an anti-inflammatory M2like-type phenotype. We are notably interested in the role of metabolic disturbances on macrophage polarization. To this end, Alienke developed a metabolic labelling method with ^{13}C-glucose to study the regulation of histone acetylation in a dynamic manner. Next to this, Sara is working on different (metabolic) stimuli to induce one or the other phenotype. Based on these cellular models we started to evaluate different lysine deacetylase (KDAC) inhibitors (see Figure 14). As readouts, 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. In addition, we have started to analyse some of the key metabolites that are likely involved in macrophage polarization.

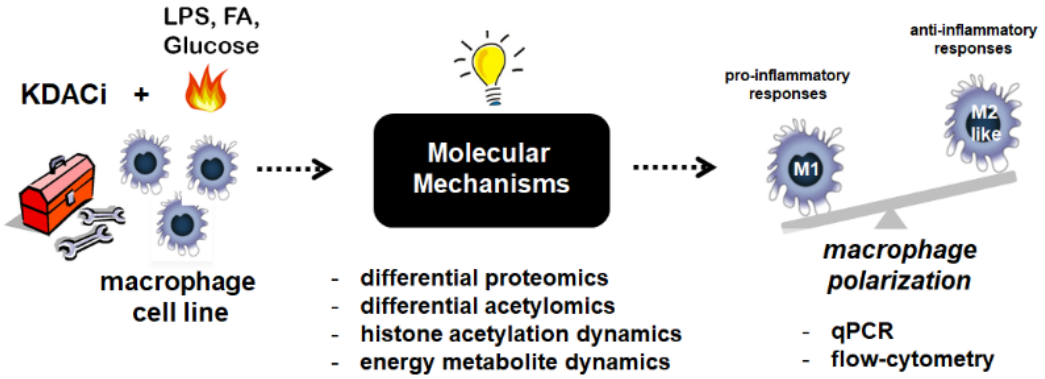

Figure 14: Scheme of the research concept. LPS: lipopolysaccharide, FA: fatty acids

7.1. Site-specific histone acetylation dynamics and simultaneous proteo-metabolomics.

In her PhD project, Alienke van Pijkeren developed 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 a tandem MS method that allows to differentiate between acetylated histone species that are isobaric at the MS1 level, thereby achieving true site-specific acetylation dynamics. Alienke showed that the combinatorial use of metabolic and chemical stable isotope labeling enables the determination of site-specific acetylation and deacetylation rates, and thus a comprehensive description of acetylation dynamics. Alienke is currently finishing a manuscript.

In addition, Alienke established a simultaneous proteome and metabolome extraction method, which is based on liquid-liquid extraction using $CHCl_3$-MeOH. She investigated the influence of different buffer systems on access to the proteome composition of the protein pellet and how they differ from conventional direct proteome extraction methods. We further established and validated the quantitative analysis of polar-ionic metabolites and free amino acids by ion-chromatography (IC)-single ion monitoring-MS (IC-SIM-MS) and LC-PRM-MS using mixed-mode chromatography. Alienke further applied the simultaneous proteo-metabolomics approach to investigate the regulatory interplay between energy metabolism and proteome in the context of tuberous sclerosis complex (TSC) disease. Currently, she is finalizing a manuscript about the evaluation of the $CHCl_3$-MeOH liquid-liquid extraction for simultaneous proteo-metabolome analysis.

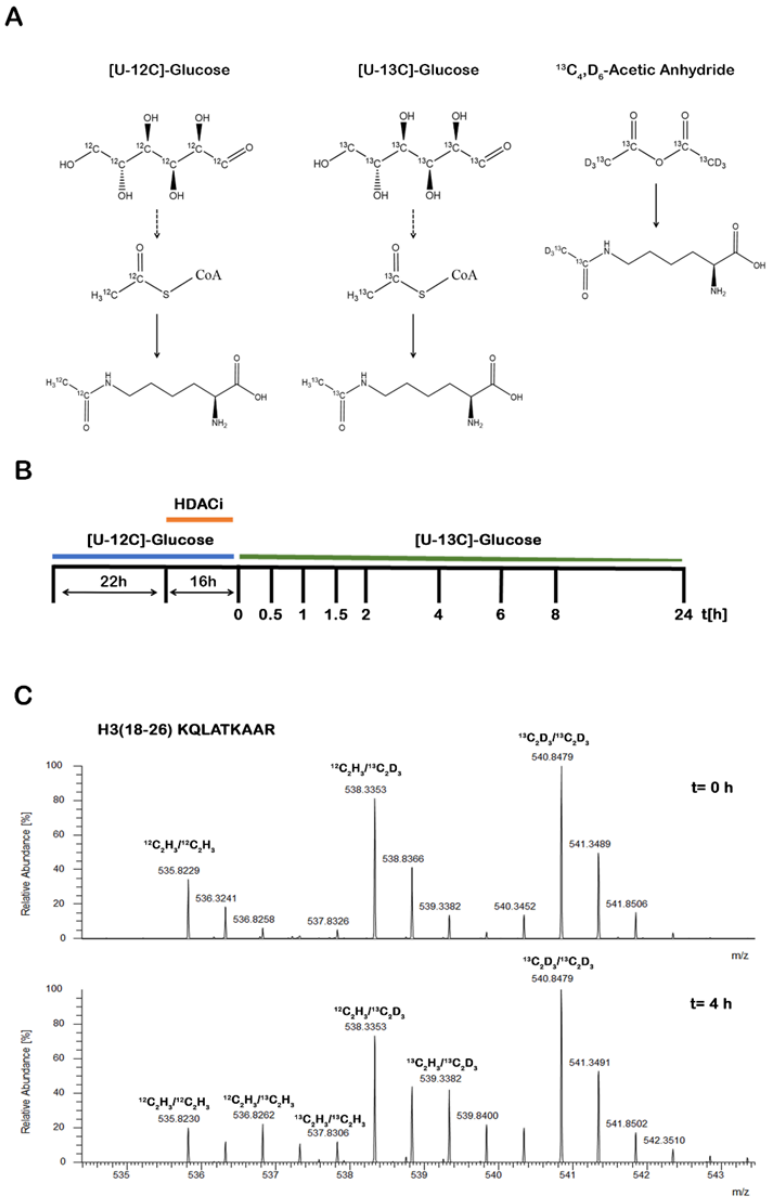

Figure 15: Incorporation of newly synthesized acetyllysine residues into different histone species (panels A and B) and site-specific quantification at the MS2 level (C). ac: endogenous acetyllysine, ac^{13}C: acetyllysine with an acetyl-group obtained from a ^{13}C-labeled tracer molecule (here $^{13}C_6$ 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. Inflammatory macrophage responses and macrophage polarization are investigated by qPCR, flow cytometry and ELISA. We are currently investigating both proteome as well as metabolome changes in relation to macrophage polarization and will apply the methodology developed by Alienke to study possible regulatory mechanisms based histone-acetylation dynamics.

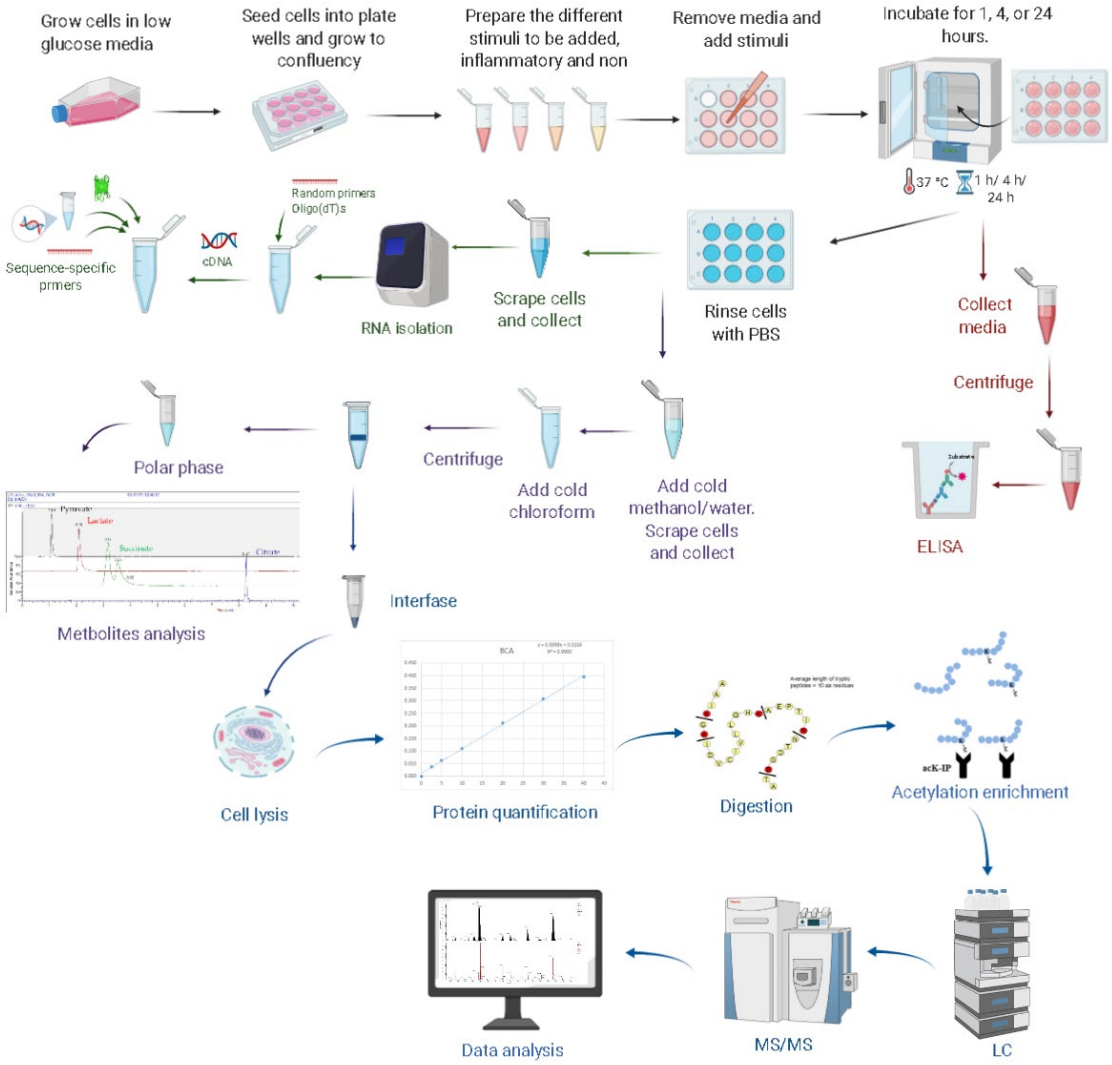

Figure 16: Workflow used to investigate the changes in energy metabolism and protein acetylation in a cell culture system (currently RAW264.7 macrophages are being used).

8. Interfaculty Mass Spectrometry Center (IMSC)

The IMSC has of course been affected by the COVID19 restrictions in the same way as other labs and facilities. The lab closure in March interrupted many running projects. The IMSC reopened in May and fortunately experienced limited instrument downtime. The IMSC has since been functioning at about 75% capacity. Some restrictions still applied until the end of the year in terms of lab access for customers and presence of lab staff.

On the bright side, equipment investments in the IMSC have continued in 2020. Notably two new LC-MS systems were installed, namely the Exploris 480 and the TSQ Altis. Both systems are aimed at proteomics applications; the Exploris 480 is a very-high resolution quadrupole-Orbitrap mass spectrometer employed for high-end untargeted proteomics projects. Next to the standard nano-LC it has a FAIMS ion mobility source that allows for further separation and selectivity of ions which should enhance sensitivity of peptide detection. The first new application that was explored is data-independent acquisition (DIA) which should allow more comprehensive proteome coverage without loss of sensitivity and with enhanced reproducibility for quantitative applications. Targeted quantitative proteomics is performed on the new TSQ Altis, also sporting the nano-LC and the FAIMS source. Development of new methods is done by and in close

collaboration with Karin Wolters & Ydwine van der Veen and the new proteomics group at ERIBA led by John LaCava. After the upgrades from 2019 we are now in an excellent position to continue providing high-level omics service for the years to come.

Figure 17*: New Exploris 480 (left), TSQ Altis (middle) and Bravo (right) equipment*

The process for acquisition of a liquid-handling platform was concluded in 2020; the Agilent Bravo system was selected, which is expected to be installed in the beginning of 2021. It is capable of both automated, high-throughput liquid-handling and sample purification, and should benefit a variety of applications including proteomics & metabolomics sample preparation.

While the number of analyses performed at the IMSC for customers has been affected by the COVID19 restrictions, the scope of the analysis remained similar covering both proteomics and small molecule quantitation, as well as analysis of among others lipids, intact proteins, vitamins & cofactors, organometallic complexes, amino acids, and numerous synthetic molecules. RUG and UMCG projects account for almost equal amounts of service work, with a small number of projects for companies. The prospects for 2021 look good with plenty of work to make the best use of the new equipment and update and streamline protocols for our standard proteomics workflows. Work to implement untargeted metabolomics, metabolite flux analysis and data independent proteomics are some of the focus points. All this work is done in collaboration with various research groups, and this synergy is indispensable for the IMSC to continue to offer state-of-the-art MS service.

Ph.D. projects

Jiaying Han (CSC scholarship)
Multiplex targeted imaging of biomolecules in tissue with high spatial resolution using laser desorption/ionisation mass spectrometry
Promotor: Peter Horvatovich
Defense: 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 (thesis expected to be defended in 2021)

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

Yanick Paco Hagemeijer
Proteogenomics data integration for the X-Omics initiative
Promotor: Peter Horvatovich
Start: February 2020

Theses

Abushareeda, W. (2020). Screening of doping substances in Human Urine with Gas and Liquid Chromatography Coupled to High-Resolution Mass Spectrometry. University of Groningen. https://doi.org/10.33612/diss.131230681

Han, J. (2020). Bioconjugation of metal-based compounds for targeted biomedical applications: from drug delivery to mass spectrometry imaging. University of Groningen. https://doi.org/10.33612/diss.113122575

Scientific Output

Scientific publications (peer-reviewed)

Al-Jaal, B.A., Latiff, A., Salama, S., Barcaru, A., Horvatovich, P., Jaganjac, M. (2020). Determination of multiple mycotoxins in Qatari population serum samples by LC-MS/MS. *World Mycotoxin Journal*, 13(1), 57-65.

Al-Menhali, A.S., Banu, S., Angelova, P.R., Barcaru, A., Horvatovich, P., Abramov, A.Y., Jaganjac, M. (2020). Lipid peroxidation is involved in calcium dependent upregulation of mitochondrial metabolism in skeletal muscle. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1864, 129487.

Alipour Najmi, A., Xiao, Z., Bischoff, R., Dekker, F.J., Permentier, H.P. (2020). Electrochemical N-demethylation of tropane alkaloids. *Green Chemistry*, 22(19), 6455-6463.

Brandsma, C-A., Guryev, V., Timens, W., Ciconelle, A., Postma, D.S., Bischoff, R., Johansson, M., Ovchinnikova, E.S., Malm, J., Marko-Varga, G., Fehniger, T.E., Berge, M. van den, Horvatovich, P. (2020). Integrated proteogenomic approach identifying a protein signature of COPD and a new splice variant of SORBS1. *Thorax*, 75(2), 180-183.

Bults, P., Sonesson, A., Knutsson, M., Bischoff, R., Merbel, N.C. van de. (2020). Intact Protein Quantification in biological samples by liquid chromatography – high-resolution mass spectrometry: Somatropin in rat plasma. *Journal of Chromatography B*, 1144, 122079.

Eriksson, O.J., Brotons, A.S., Rezeli, M., Suits, F., Markó-Varga, G., Horvatovich, P. (2020). MSIWarp: A General approach to mass alignment in mass spectrometry imaging, *Analytical Chemistry*, 92 (24), 16138–16148.

Faassen, M. van, Bischoff, R., Eijkelenkamp, K., Jong, W.H.A. de, Ley, C.P. van der, Kema, I.P. (2020). In matrix derivatization combined with LC-MS/MS results in ultra-sensitive quantification of plasma free metanephrines and catecholamines. *Analytical Chemistry*, 92(13), 9072-9078.

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Yang, K., Mesquita, B., Horvatovich, P., Salvati, A. (2020). Tuning liposome composition to modulate the corona forming in human serum and uptake by cells. *Acta Biomaterialia*, 106, 314-327.

Articles on preprint servers

Bernal, V., Bischoff, R., Horvatovich, P., Guryev, V., Grzegorczyk, M. (2020). The 'Un-Shrunk' Partial Correlation in Gaussian Graphical Models, DOI: 10.21203/rs.3.rs-76682/v1 [under review in BMC Bioinformatics].

Feng, X., Zhang, W., Kuipers, F., Kema, I., Barcaru, A., Horvatovich, P. (2020). Dynamic binning peak detection and assessment of various lipidomics liquid chromatography-mass spectrometry pre-processing platforms, bioRxiv 2020.10.10.334342; doi: https://doi.org/10.1101/2020.10.10.334342 [under review in Analytica Chimica Acta].

Peer - reviewed conference articles (Proceedings)

Bernal, V., Guryev, V., Bischoff, R., Horvatovich, P., Grzegorczyk, M. (2020). *Correction for the shrinkage effect in Gaussian graphical models*. 281-284. Paper presented at 35th International Workshop on Statistical Modelling, Bilbao, Spain.

Bernal, V., Guryev, V., Bischoff, R., Horvatovich, P., & Grzegorczyk, M. (2020). *Uncertainty propagation in shrinkage-based partial correlations*. 285. Paper presented at 35th International Workshop on Statistical Modelling, Bilbao, Spain.

Lectures

Horvatovich, P., Challenges of proteogenomics data integration and LC-MS/MS pre-processing to study complex diseases, online 1st International Week Symposium of University San Francisco, "Comprehensive clinical research: from bench to bedside", November 5, 2020.

Merbel, N.C. van de, Supporting the development of protein drugs by LC-MS-based bioanalysis. PRA webinar, October 6, 2020.

Merbel, N.C. van de, Recent developments in LC-MS-based bioanalysis of biopharmaceutical drugs. AAPS Pharm Sci 360 on-line symposium, October 27, 2020.

Merbel, N.C. van de, The best of both worlds: developments in hybrid ligand-binding / LC-MS approaches for peptide and protein quantification. European Bioanalysis Forum, 13th Annual Open Symposium, on-line, November 18, 2020.

Tian, X., Vries, M. de, Permentier, H., Bischoff, R., A collision-induced dissociation cleavable isobaric tag for peptide fragment-ion-based quantification in proteomics. CHAINS 2020 (The Netherlands), online meeting, December 7-9, 2020.

Bioinformatics source code

MSIWarp mass spectra alignment tool for mass spectrometry imaging data. https://github.com/horvatovichlab/MSIWarp

Poster presentations

Alipour Najmi, A., Xiao, Z., Bischoff, R., Dekker, F.J., Permentier, H.P., 2020. Electrochemical N-demethylation of tropane alkaloids. CHAINS 2020, online meeting, December 7-9, 2020.

Tian, X., Vries, M. de, Permentier, H., Bischoff,R., Selective maleylation directed isobaric peptide termini labelling for accurate proteome quantification. 53rd Annual Conference of the DGMS Including 27th ICP-MS User Meeting, Münster, Germany, March 1-4, 2020.

Tian, X., Vries, M. de, Permentier, H., Bischoff, R., A versatile isobaric tag enables proteome quantification in data dependent and data independent acquisition mode. HUPO Connect 2020, online meeting, October 19-22, 2020.

Tian, X., Vries, M. de, Permentier, H., Bischoff, R., A versatile isobaric tag enables proteome quantification in data dependent and data independent acquisition mode. CHAINS 2020, online meeting, December 7-9, 2020.

Workshop organization

Horvatovich, P., X-Omics workshop on “Data integration and standards”, June 18, 2020.

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

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

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

Teaching

Academic Research & Communication Skills 1, WPFA18001, essay & poster mentoring	February – June 2020
Academic Research and Communication Skills 2, WBFA003-04, presentations & minithesis mentoring, lecture	September – October 2020
Academic Research & Communication Skills 1, WBFA001-05, scientific paper analysis, mentoring	November 2020
Quantitative Bioanalysis (WMFA14005)	February, 2020
FATEM WLFB1210, lab tour and lectures	February 16-20, 2020
Prominent PhD course, Proteogenomics and personalized medicine	June 04, 2020
Data Science in Biomedicine (WMBM023-05), Practical for Proteomics in R session	October 05, 2020
BMS from Big Data to Personalized Medicine (WMBM008-05), Proteomics applications for personalized medicine	September 24, 2020
Bachelor thesis & projects	February – March 2020
Biostatistics (WLFB1001 in 2019/2020 Ib and WBFA011-05 in 2020/2020 Ib)	November 13, 2019 – January 21, 2020 and November 9 2020 – December 1, 2020.
Bioanalysis (WBFA19004)	June 02 – July 10, 2020
Instrumental Analysis, lectures & QA session	April 30 & May 01, 2020
Drug Development (masters)	September 07, 2020
MMIT lecture (Top class 2)	December 07, 2020
Molecular and Cellular Neuroscience (MLBCNN07)	November 19, 2020
Mass Spectrometry (open course)	December 10 – 11, 2020

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

March 12, 2020, Teaching in “Master of medicament” at University of Strasbourg, Faculte de Pharmacy (online) course “Introduction à la protéomique”.

Student projects

- Jessica Alferez del Castillo, November 08, 2019 – May 18, 2020, IMI international master’s project: Proteomic profiling in MCAD: towards patient risk assessment. Supervisor: Dr. Karin Wolters
- Victoria Aboagye, start November 09, 2020, MPS master’s project: Deamidation of asparagine in the Complementary Determining Region of (CDR) Trastuzumab in complex with Her-2. Supervisor: Baubek Spanov
- Ikram Yacheur, start February 10, 2020, bachelor project Hanze University: The effect of macrophage stimulation on cytokine levels in relation to the development of Diabetes Mellitus Type II and Chronic Obstructive Pulmonary Disease. Supervisor: Sara Russo
- Dominique ter Maat, start September 07, 2020, bachelor project Hanze University: Extracellulaire vesikels als potentiële biomarkers voor, door medicijnen ontwikkelde, cholestase. Supervisor: Janine Stam
- Rutger Schipper, Feb-March 2020, Pharmacy, project: The synthesis and purification of acetyl-alanine-p-nitrophenol and acetyl-cysteine as chemical tags to label peptides for quantitative proteomics; co-supervision with Xiaobo Tian

Individual teaching

- BMS Colloquium (Victor Pera, Brenda Baak)
- Bachelor Pharmacy assay and projects (Ymke van der Veen, Rob Friedrick, Cees Boone, Emma Ruiter, Ziad Aziz) on the topics of “Proteogenomic data integration”
- Bachelor Chemistry of Life Alex Skvortsov (40% supervision).
- MPDI master projects: “Thermal proteomics-based target landscape of Ibrutinib in childhood B-cell acute lymphoblastic leukemia” (Anastasia Audrey) and “Identification and Functional Characterisation of DNA Insertions in Genomes of Head and Neck Cancer Patients Using Proteogenomics Approach” (Daria Vedernikova)

Outlook

2021 will see a major change in the Department of Analytical Biochemistry (AB) and the Interfaculty Mass Spectrometry Center (IMSC). Prof. Dr. Rainer Bischoff will take his well merited retirement at the end of 2021. Rainer joined the University of Groningen in 2001 leaving a group leader position at Astra Zeneca in Lund, Sweden and built up the AB Department and became the scientific head of the IMSC. In these years, he built an outstanding research line in biomarker discovery and development, multi-omics analytical profiling, targeted and untargeted biomolecular analysis and participated in many clinical studies leading to 300 articles and other types of scientific output. Amongst others, Rainer developed, with colleagues at AB and IMSC, an automated analysis platform for activity profiling of metalloproteases, performed the successful discovery and validation of biomarkers for the early detection of cervical cancer and developed electrochemical and chemical labeling methods for specific biomolecule sensing. Under his leadership, AB and IMSC received funding from the Netherlands Bioinformatics and Proteomics Centers (NWO), The Dutch Cancer Society (KWF), The Dutch Technology Foundation (now NWO-TTW) and the European Commission, and was amongst the founders of the Biomarker Development Center. After his retirement he is planning to actively contribute to the scientific work of AB and IMSC by mentoring and supervising his current PhD students. Rainer also contributed to the academic education of multiple generations of students by teaching amongst others the Pharmaceutical Analysis C (Bioanalysis this year) course in the Pharmacy bachelor curriculum and contributed to other courses such as Drug Development: from Design to Evaluation, Instrumental Analysis, Molecular and Cellular Neuroscience and Quantitative Bioanalysis.

Our future activities, besides continuing our original research lines of computational mass spectrometry, biopharmaceuticals, biomarkers and electrochemistry-mass spectrometry will be determined by a, to be hired, new researcher expecting to perform mass spectrometry based multi-omics research and by further integration of the IMSC into the network of Research Core Facilities initiated by the Dean and Vice-Dean of the Medical Faculty. This will, over the longer run, lead to more concerted efforts to provide cutting-edge enabling technologies to all researchers at the Medical Faculty and the Science and Engineering Faculty 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. John LaCava, a new PI who started in ERIBA focusing his research on protein-protein interactions with heavy use of mass spectrometry techniques, is joining the work at the IMSC. His contribution opens new opportunities for funding and allows joining forces in state-of-the-art mass spectrometry method development.

Peter Horvatovich took a more distinct role in defining the research strategy of the Department in recent years, will continue to act in this role in the future. Peter has developed into a very successful, independent researcher with a wide network of national and international collaborations such as the Chromosome Centric Human Proteome Project (C-HPP). He is actively involved in the Swedish arm of the worldwide Cancer Moonshot Project (http://www.cancermoonshotlund.com/) initiated by Joe Biden to achieve a breakthrough in cancer treatment. This collaboration allowed him to develop and apply his extensive knowledge in data processing, biostatistics and data integration, to advance the understanding of molecular mechanisms of melanoma development and metastasis and develop new personalized diagnostics and treatments. 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 is opening new avenues for research at the Analytical Biochemistry Department in Groningen with new collaborations on the detection of intact drug distribution in tissue with Daan Touw (UMCG).

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. This year we have installed new high-resolution and targeted/quantitative LC-MS equipment, liquid handling robotics and extended our computational infrastructure with 2 high-performance computational clusters for proteogenomics data integration jointly with Victor Guryev (ERIBA, UMCG). We hope that the purchase of a new high-resolution instrument supporting metabolomics work will be possible this year.

The COVID-19-pandemic-related restrictions from March 2020 made work at the laboratory and teaching as well as traveling to conferences and workshops very challenging. We have been able to join forces to adapt to the situation and organize the work and teaching in an efficient way as best as possible allowing master students, PhD students and postdocs to continue their lab work despite some delay. In light of the available vaccines, we hope that the next academic year can be started without or with very few restrictions.

With this, we 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