



Customer Insights

- Traversing Omics: How Mass Spectrometry Advances Translational Medicine

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Bruker's unique set of mass spectrometry hardware and software solutions enable advances in personalized healthcare at the Radboud university medical center.



Professor Alain van Gool, Professor of Personalized Healthcare, benefits from a unique relationship with Bruker and specialized mass spectrometry instruments for translational research.

"Bruker really takes the lead as mass spectrometry technology providers – they facilitate our key objective of making a real impact on healthcare and help us put patients at the heart of our translational research."

Translational Metabolic Laboratory, Radboud University Medical Centre

Radboud university medical center (Radboudumc) in Nijmegen, Netherlands, is a large medical school of approximately 11,000 employees, containing 50 departments which collectively focus on personalized healthcare, and whose core activities are centered on patient care, research and education. The Radboudumc's technology infrastructure is organized through 19 Technology Centers that span omics, imaging and data analysis to name a few, and are coordinated by Professor Alain van Gool. The Technology Center Mass Spectrometry includes multiple laboratories that use mass spectrometry (MS) to support their activities including Pharmacy, Pharmacology & Toxicology and the Translational Metabolic Laboratory.

The Translational Metabolic Laboratory (TML) that is headed by Professor van Gool employs about 120 people and serves as a mixed-function diagnostic research laboratory. Research into the metabolic basis of disease mechanisms is translated into biomarkers and validated diagnostics. TML bases a large part of its

technological approach on MS through integrated proteomics, glycomics and metabolomics mass spectrometry platforms. TML is part of the larger Department of Laboratory Medicine, that together with 4 other clinical laboratory departments, Genetics, Pathology, Pharmacy, Medical Microbiology, are integrating to form one large Radboud Diagnostic Laboratory with approximately 1200 employees.

Professor van Gool describes his laboratory's role at Radboudumc:

"I'm responsible for the Translational Metabolic Laboratory, an enthusiastic group of 100+ translational scientists that work intensely with clinicians and molecular researchers to understand human disease and translate such research into novel personalized diagnostics."

The work

Professor van Gool's laboratory at Radboudumc specializes in the metabolic side of disease, focusing on proteins, metabolites and enzyme functions, and combine this with insights from Radboudumc's genetic analysis to deliver comprehensive molecular diagnostics. Key application areas are genetic

metabolic diseases, mitochondrial diseases, iron deficiencies, and neurological disorders. Being a diagnostic laboratory, the key customers are clinicians from Radboudumc or surrounding hospitals requesting tests. The re-imbursement of such tests from health insurance providers, supplemented with research grants, enables them to operate as a clinical diagnostic laboratory. The laboratory's main objective is to make an impact on healthcare, and so even though they do open up for external users, Professor van Gool does not commercialize the diagnostic tests as marketed products.

The Translational Metabolic Laboratory has been developing comprehensive analytical workflows based on mass spectrometry and have implemented several in diagnostics. *We are now moving to the next phase of omics-based diagnostics in which we detect a large collection of metabolite or protein features that help us to do a first passage diagnostic clustering, after which the identity of the differential biomarkers support an in-depth mechanistic interpretation. Of special interest is the stewardship of the generated data, which on one hand should be closed to maintain patient confidentiality and on the other hand should be open to share with the scientific community. This requires a thorough data architecture with well-defined procedures for sharing.*" explains Professor van Gool

Data analysis is gaining importance in a diagnostic omics laboratory like the Translational Metabolic Laboratory. More specifically, one can consider three levels of data analysis undertaken by the laboratory. In the first stage, the raw mass spectrometry data first has to be converted into an analyzable

format. The second level is determining differential signals between samples as a result of a treatment or a disease, including coupling of genomic output with a proteomic output. Professor van Gool describes the third level:

"The third level is the biological investigation: what does it mean? For instance, we may discover a differential glycosylation that we didn't know about before. This requires a follow-up biological investigation which is a different skill set altogether. The first stage uses vendor software to go from raw data to a pre-processed data set; the second stage is partly vendor data but uses chemometric pipelines as well (mostly self-programmed). We use a combination of those to build a whole workflow, resulting in a shortlist of validated, confirmed differential patterns that we see. We do this with our metabolomics, glycomics, and proteomics data. The third stage involves looking with the scientists at the biology, supported by statistical differential analysis with the second level."

Glycoproteomics

Post-translational modification (PTM) occurs during or after protein biosynthesis with a wide variety of possible outcomes. For example, phosphorylation introduces a new phosphate group to the protein chain, a crucial event in cellular signal transduction. Glycosylation results in the attachment of a new carbohydrate molecule to a protein. Approximately 80% of all proteins are glycosylated, many of which are major determinants of health and disease: around half of all human diseases are affected by glycosylated



proteins. PTMs, therefore, are highly relevant disease biomarkers and can be experimentally detected using the Bruker maXis™ 4G ETD.

“To be able to look at glycosylated proteins, you need extremely high resolution hardware so that you can distinguish the different types of carbohydrate chains (the glycosylations) with high accuracy, so there is no doubt over the nature of the glycosylation branch.”

“Also, you need to be able to annotate the different peaks with complementary software to automatically identify the glycosylation pattern. In both areas, Bruker is really strong. Their software really goes hand-in-hand with the hardware for glycoanalysis”

explains Professor van Gool. His group has been studying glycosylation mechanisms in recent years, to elucidate which mutations in patients affect the mechanisms of glycosylation. Dirk Lefeber, Associated Professor of Glycosylation Disorders working in the Translational Metabolic Laboratory and in the Department of Neurology, has done groundbreaking work to acquire mechanistic insights in glycosylation disorders. Together with Monique van Scherpenzeel,

glycosylation scientist in TML, Dirk translated this knowledge into diagnostic assays for intact glycoproteins and glycans. In collaboration with Hans Wessels, a proteomics scientist in TML, the glycosylation and high throughput proteomics approaches were combined, resulting in a glycopeptide profiling workflow with very promising diagnostic potential.

“We now can detect around 40,000 unique glycopeptides in a single MS scan.”

Our recent cohort analysis show that such profiles can be used to cluster patients based on their unique profile with unparalleled specificity.

“This is only possible if you have the right hardware and software, and our partnership with Bruker contributed strongly to this new method”

comments Professor van Gool, “We invested heavily in chemometric pipelines last year, to cope with analyzing the data resulting from these workflows. Using these pipelines, we can use the whole profile – about 1 million data points – to cluster patients into certain groups.



If you can identify the defect or mutation in those clusters, that's a very quick diagnosis. The next step we're taking is to actually identify all those differential glycopeptides, to really break it down to single biomarkers, which helps us to identify the mechanism of disease and develop novel personalized diagnostics.

"That's a promising foresight towards translational medicine!"

Protein glycosylation profiles can be changed following mutations in the genome, but also if the patient is challenged with certain disease or infection. *"A comprehensive method such as glycopeptide profiling allows us to take a system biology approach in diagnostics. Many*

people in the field just focus on one or a few known suspect glycoproteins, which only looks at a very small part of the puzzle. We strongly believe in a more holistic approach that complements the genomic diagnostic analysis. For us, metabolomics has led the way in such functional omics diagnostics, glycopeptidomics is probably next, and ultimately I would like to go to the intact glycoprotein level."

"That will be the third layer. We have excellent translational scientists such as Dirk, Monique, Hans and many others in our omics group, who can make that happen" explains Professor van Gool.

Intact protein analysis

"I've been working in the biomarker field for quite some time now, in Pharma, research institutes and now in academic hospital, and over the years

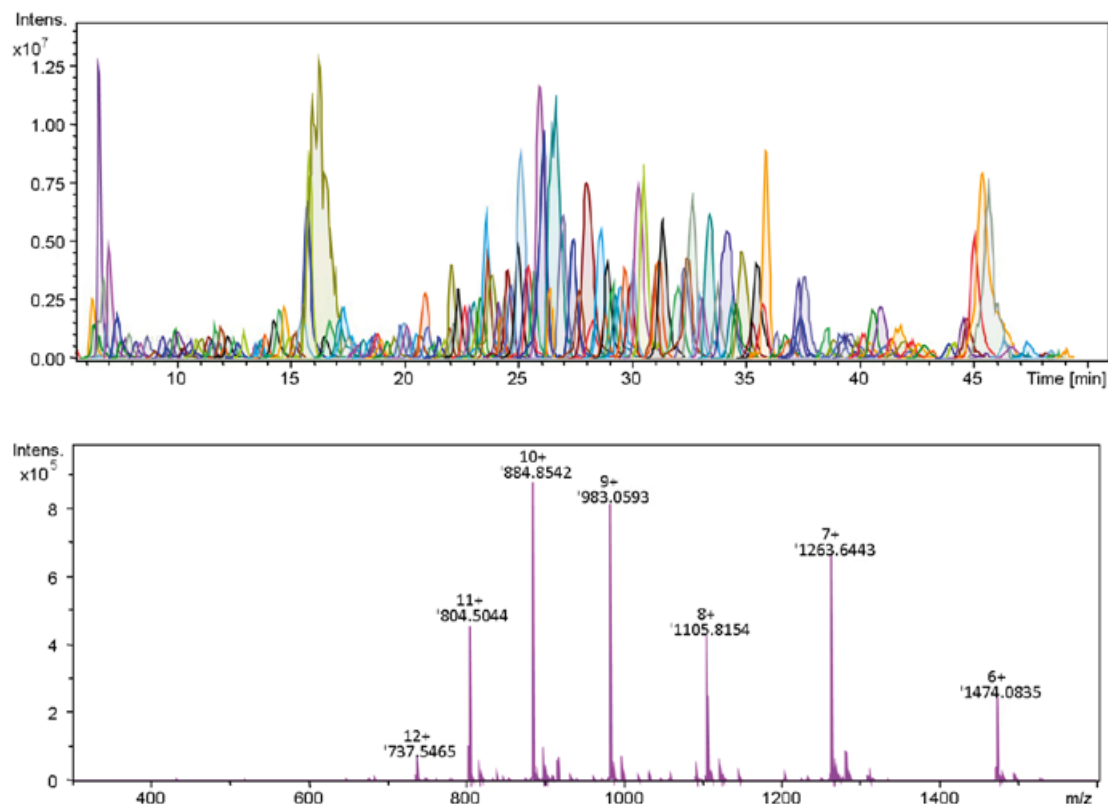


Figure 1: Proteoform profiling in Bovine heart mitochondria by Top-Down proteomics. Top: High resolution liquid chromatography separation of >2900 isotopically resolved intact mitochondrial proteins in >200 dissect feature chromatograms. Bottom: ESI-MS spectrum and charge deconvoluted spectrum insert for feature 119 which is the 8838.46 Da proteoform of mitochondrial complex V subunit ATP5J.

I've found it very surprising that we talk about the complexity of the genome into proteomes – we already know that each gene leads to about 20 different proteoforms – while we ignore that in diagnostics. We routinely use an immunoassay to detect a small epitope (the part of an antigen recognized by the immune system) of about six amino acids and we just ignore the rest of the protein that may have huge effects on its activity. I find this very strange. The best way is to look at the whole protein, if we can" explains Professor van Gool.

Technology has thus far restricted the analysis of intact proteins. However, in recent years this has been developed and top-down proteomics is gaining strength in laboratories, such as that at Radboudumc, for application in intact protein analysis, PTMs and analysis of low to medium complexity samples. Hans Wessels specializes in the development and application of top-down proteomics and protein complexes, and uses the Bruker maXis™ 4G ETD for his work.

"We use Bruker instruments, supported by the software, to look at a purified complex where we can identify about 240 different proteoforms from the 40 subunits."

"We're still in the research phase, but we're

able to isolate different protein complexes in the mitochondrial energy chain in mice, and easily identify the different complex compositions in different tissues – this was a pleasant surprise! This opens up an entirely new level of biology, which is extremely interesting. The plan is to take this further to diagnostics" comments Professor van Gool.

For example, proteins can be modified differently in one tissue than they are in another. Those modifications can be taken on an inter-protein level forwards as a diagnostic tool. This methodology is currently available for studying intact proteins for single proteins, protein complexes and biopsies. Professor van Gool's laboratory is moving towards analyzing proteins from body fluids:

"We've done interesting pilot studies in plasma and cerebral spinal fluid (CSF), where we analyzed proteins under 30kDa in size on an intact protein level and were able to see thousands of intact proteins, and could identify them, including their modifications. This is a very interesting approach. In the brain CSF, most of the signaling and activity is mediated through peptides. So you don't have to digest the proteome, you can just look at intact proteins."

"We're now working with Bruker to develop the optimum hardware and software to distinguish all the multi-charged peptides, to identify then using CID and ETD."

We can then use the follow-up pipeline on the differential analysis, which we have built ourselves. This is still early days but conceptually, I really believe in this approach, rather than looking at small peptides."



Harnessing genomics

Professor van Gool's Translational Metabolic Laboratory works closely with the Department of Genetics at the Radboudumc, where approximately 8000 patients are genetically investigated in a diagnostic setting by whole exome sequencing. From 2018, the department is moving towards whole genome sequencing and will combine these efforts with the functional omics efforts at TML.

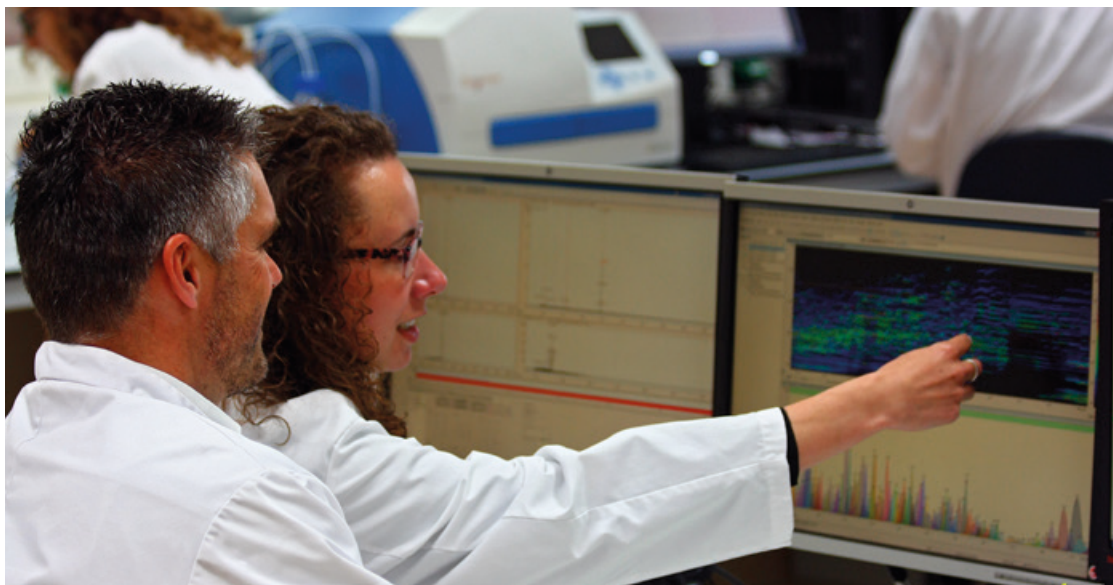
"Sequencing patients is a powerful approach towards a personalized genetic diagnosis, but after filtering a lot of variance away you may still have 10-20 variants remaining – you have to pick which one is the causal mutation. We support this with functional omics platforms to contextualize those mutations, so together we can make a thorough personalized diagnosis. This tandem approach with next gen sequencing and mass spectrometry is extremely powerful, and one which we believe is completely unique to our academic medical center."

The Department of Genetics at Radboudumc begun diagnostic whole exome sequencing in 2013, and have been growing their capabilities since to the current throughput of 8000 exomes a year.

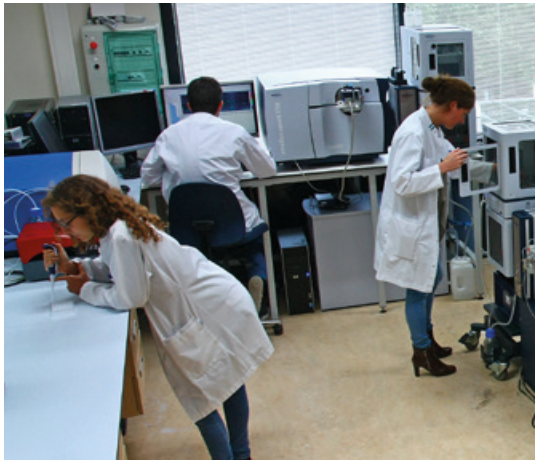
Adding the extra layer of metabolomics is complex, but helps scientists uncover which variant is associated with a certain clinical phenotype.

"In the years to come, I think this approach will help us make better diagnoses – it really is a complementary technology..."

...explains Professor van Gool, "we like to believe we're pretty good at genetic screening of patients – we do this successfully in our Department of Genetics – but now we're adding this next generation layer of functional omics to this whole genetic screening. We see this as a strategic way of moving forward, to really interpret patients, not by single mutations but as the system we are. Ultimately we want to have functional omics pipelines, where genetics is followed by metabolomics in cases where we suspect a metabolomics difference."



"We also want to combine genetics with glycoproteomics where we suspect there is something happening in the system."



Challenges and solutions

One of the key challenges facing the personalized healthcare industry, and Professor van Gool's laboratory, is the innovation gap in biomarker research and development.

Thousands of novel candidate biomarkers are in our discovery research, without proper follow-up experimentation to validate findings and to develop them to improved robust diagnostics. "To illustrate, approximately 2000 new biomarkers for prostate cancer are published per year in peer-reviewed journals, which equates to approximately 5 per working day. It takes 1-3 years to validate a biomarker and 1-10 years to develop a diagnostic assay, so the process is out of balance. This is putting extra pressure on our translational medicine field" comments Professor van Gool.

To overcome such gaps, several recommendations can be made. One of these focusses how we deal with our research data. Good data stewardship applies the FAIR principles to all experiments. Professor van Gool explains how this should be implemented:

"FAIR stands for Findable, Accessible, Interoperable and Reusable – this is a very important principle which, if implemented properly, encourages the production of data which can be found and re-used by other scientists, using accurately documented methods."

"This way of working will certainly contribute to improving the reproducibility of scientific studies."

"It is fundamental way of working in which we aim to share our data for the greater good of science, to enable that together we can do better science than by ourselves. In addition, I believe we should be more critical to ourselves as scientists to ensure we strive for highest scientific quality (i.e. reproducible research with impact) and focus less on quantity (mere number of publications). For instance, we should ask ourselves whether the sample number is high enough for statistical analysis. Is the chosen technical approach the best suited to yield answers of the end user? Should you first do a pilot study to determine the variables in our experiment? We have to think more about the end use of your output data. We're putting a lot of effort into boosting these principles, nationally and internationally."

To overcome such challenges, Professor van Gool explains how his former laboratory in the pharmaceutical industry worked by 'killer experiments':

"We developed an approach, optimized the method, then we tested the crucial set of patient samples to prove the added value of the diagnostic method. If the approach was insufficient, we killed it. It is not very academic, but it works!"

He now translates this to the novel potential diagnostic methods within the Translational Metabolic Laboratory. For example, for the glycopeptide profiling approach, a successful proof-of-principle experiment was done in which the full profile of glycopeptides could strongly distinguish patients with 4 different congenital defects in glycosylation from each other and healthy controls. Subsequently, the laboratory has recently performed a screening of 200 patients, representing different known disorders of glycosylation, unknown mechanisms of disease and control patients, with the aim of defining a pattern with substantial chemometric analyses to develop a shortlist of biomarkers in order to distinguish those 14 disease mechanisms by one set of glycopeptides. Also, a large number of controls were included to demonstrate the robustness of the method. The optimized glycopeptide profiling assay has 1 million data points representing over 40,000 unique glycopeptides in one run, derived from a Q-TOF profile on the Bruker maXis™, on which a chemometric filter is applied – based on a genetic algorithm random forest– to further develop this profiling method into a robust test to diagnose patients with genetic metabolic disorders.

“We asked ourselves, can we take those complex data, develop the algorithm, and take a sample to test and prove that it works. If we couldn’t, we’d kill that approach, use it as a discovery tool and try something different. We do seem to be able to do this in a reasonable timeframe, however, with a fast turnaround time and a confidence level greater than 99%.”

“We consistently get high confidence with single biomarkers, and get extremely good selectivity and specificity using panels of such biomarkers.”

“Particularly the combination with the genetic analyses is really important for our metabolic diagnostic laboratory, demonstrating the value of multi-omics approaches.”

Collaboration

“Everything we do is through collaborations” explains Professor van Gool, “we work in a kind of collaborator network with the different Technology Centers at Radboudumc, as well as with vendors, clinicians and particularly, with the Department of Genetics to create our pipelines. We also work with the experts in chemometrics and bioinformatics, to ensure we end up with one streamlined process. The combined clinical laboratories at Radboudumc have 1200 employees that work as one large team with each individual carrying specialist knowledge on specific areas.”

Outside of the Radboudumc, the laboratory collaborates with the Dutch Techcenter for Life Sciences (www.dtls.nl), which groups different



technologies into 120 'Technology Hotels'. Also, the laboratory is part of EATRIS, the European infrastructure for translational medicine (www.eatris.eu) in which Professor van Gool chairs the Biomarker Platform, and of specific proteomics, glycomics and metabolomics communities:

"Collaborating within in large scale research infrastructures such as DTL, EATRIS, Health-RI and others combines all the technologies we have with those across the networks."

"This enables us to join forces and do more than we can individually. For instance, we just filed a major grant proposal to set up the Netherlands X-omics Initiative, combining all the omics in the Netherlands."

"In Europe we collaborate with others by contributing our translational omics infrastructure. Here we have a unique position by already applying omics in clinical diagnostics, combining genomics, metabolomics, glycomics and proteomics."

Working with Bruker

Professor van Gool has been working with Bruker since 2002, when he purchased the ProteinScape 1.0 software – the bioinformatics platform for storage and processing of MS and MS/MS proteomics and glycomics data – whilst working at the pharmaceutical company Organon. The success of this first purchase then led Professor van Gool to purchase the autoflex MALDI-TOF/TOF. He describes the progression of his relationship with Bruker:



“The ProteinScape software worked extremely well for us, and the autoflex really outperformed other MALDI instruments I had in the lab at the time.”

I became more and more interested in what Bruker had to offer and really began to value the interactions I had with them. When I set up a biomarker laboratory in Singapore in 2009 I unfortunately couldn't have Bruker machinery there – so when I got back to the Netherlands the choice was pretty easy:

“If I wanted to achieve advancements in glycoproteins and intact protein analysis, Bruker was the clear choice.”

Following a successful introduction to Bruker, Professor van Gool purchased their maXis™ Q-TOF and the amaZon speed ion trap LC-MS instruments in 2014. The decision to purchase such equipment comes from a two-way partnership, where Hans Wessels data-tested instruments and software to give the people at Bruker a lot of user experience, to enable them to continue improving their products. Professor van Gool sits on the advisory board, which facilitates the continuous communication between users and vendor and enables the development of technology of particular relevance to the field.

“Having the software really brought me on board with the hardware, the data from which has received great feedback from other pharma colleagues – so the choice to stick with Bruker was a good one! I haven't been disappointed with them thus far, and I really value the collaborative spirit we have. The easy access to experts in Bremen is very helpful: it's not only the performance or price of their instruments, but the interaction with the company is a really important criteria to me.”

Future

As the field of translational medicine and personalized healthcare progresses, so are the technologies used by proteomics, glycomics, metabolomics and genomics laboratories. Since scientists realise that the full proteomics picture cannot be obtained from digested proteins, the intact approach using innovative top-down proteomics and particular focus on post-translational modifications is set to take over more laboratories internationally. Laboratories such as that of Professor van Gool bring such new technologies into the clinical setting, and use translational science to increase insights into disease mechanisms to improve personalized patient care. By directly reporting to the hospital executive board, Professor van Gool drives the future integration of translational technologies in science and medicine.

“I believe that we, as a biomedical research field, could really improve the quality of what we do. It's time for quality and not quantity in terms of biomarker discovery. This starts with the vendor, and the way we produce our data.”

“This is where Bruker supports us as a community, and help us move forwards.

It's amazing how the technology they provide us with can break down the barriers which have previously prevented the widespread adoption of techniques like top-down proteomics and translational omics.”

For more information about Bruker's mass spectrometry solutions, please visit <https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-toftof.html>. For more information about proteomics at Radboud University Medical Center, please visit <https://www.radboudumc.nl/en/research/technology-centers/mass-spectrometry/proteomics>.

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