Featuring cutting edge research projects and enabling technologies of the Netherlands Proteomics Centre and Proteins at Work
High speed

We are proud to present this first new-style edition of the NPC HighLights Magazine with a renewed design and content. The central theme in this issue is ‘Proteomics in a European Context’. Prominent scientists from various European institutes contribute to the theme where they unanimously conclude that developments in proteomics are moving forwards at a fast rate.

This ‘high speed’ is aptly expressed by Jesper Olsen. In the interview he draws the analogy with Formula One racing: “We tend to think that they cannot go any faster, but then every season there is a new car that outraces the rest.” Olsen doesn’t want to trail behind. “Let’s face it, we all want to race in the fastest machine.” Being out on top, however, needs both a fast car and a competent driver as well. Only a perfect combination of the car (mass spectrometer) and its driver (the proteomics scientist) will triumph. Striking examples of such winning combinations are illustrated in the NPC Progress Meeting report. The overview of summarised keynote lectures offers a nice view of the ongoing advances in mass spectrometry techniques and its applications in molecular biology.

Alongside the central theme and interview, various topics in the field of society, education, awards, thesis or valorisation will also be covered. In this issue specific attention is paid to the annual European Summer School, the one-day proteomics experience of primary school pupils, the new art-and-science project by Charlotte Jarvis and, finally, the role and impact of proteomics in the famous project ‘Grandiose’.

We hope that the renewed NPC HighLights Magazine may remain the specialist journal by and for the proteomics community. Enjoy!

Editorial board NPC HighLights

About

The Netherlands Proteomics Centre (NPC) is a virtual centre that serves as a platform for proteomics research. With the scientific programme Proteins At Work addressing key areas of proteomics in several projects, and specialised ‘research facilities’, the NPC performs high-quality research and provides access to state-of-the-art proteomics technology, equipment and expertise to researchers in the life sciences from academia and industry. Proteins At Work is made possible by NWO.

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For those in charge of organising (trans)national funding for research and technology, it is always sensible to consider practices in different countries. All systems have their pros and cons and the trick of course is to copy the pros and avoid the cons. In the case of Denmark however, it will be very hard to take over any element, good or bad. “Denmark has quite a special organisational structure when it comes to research funding.”

Proteomics in pole position

Novo Nordisk is one of the world’s leading healthcare companies, particularly known for diabetes care. “Most of Denmark’s leading companies are owned by foundations, which is in part motivated by tax reasons, but also offers protection against hostile take-overs. In return, these foundations invest heavily in research,” says Jesper Olsen. Government funding for research in Denmark is no match for the very deep pockets of industry. “And government funding is mostly focused on strategic research anyway,” Olsen says disapprovingly. This raises an interesting point: apparently public research funding in Denmark is limited and not focused on curiosity-driven science, leaving scientists in the hands of private parties to fund their research.

Centres of excellence
In this interplay of forces, is there room for basic science? Very much so, says Olsen. “The funding streams from the company foundations are primarily allocated towards basic science. And there is no direct link between the activities of a company and the type of research they fund and the companies are also not involved in the selection or the execution of research projects.” The foundations channel their funds mostly through grant applications to individual scientists based on open calls and peer review. Olsen: “Danish multinationals like Novo Nordisk, Lundbeck, Carlsberg and Velux make huge profits. Particularly in the case of Novo Nordisk, the budgets have expanded so much lately that the foundation decided to, next to the grants, also invest in a limited number of centres of excellence in basic science. That is how the Novo Nordisk Foundation Centre for Protein Research was set up.”

Strong basis
Because of the lack of substantial national funding for research and technology, Denmark does not have central facilities for proteomics and other high-level technologies. However, this does not mean proteomics is absent from the Danish research landscape. On the contrary, the country has a long history in proteomics. “The strong Danish position in proteomics started with Peter Roepstorff at the University of Southern Denmark in Odense. He pioneered the use of mass spectrometry for protein sequencing already thirty years ago. Because of his ground-breaking work, his lab basically acted as a national facility for proteomics and they were able to recruit world-leading proteomics researchers such as Matthias Mann. ”Starting around ten
years ago, the realisation kicked in that each university needs proteomics and since then, mass spectrometry-based proteomics research groups have arisen throughout the country. “But technology development is still mostly based in Odense and here in Copenhagen,” says Olsen, who himself got hooked on mass spectrometry during his bachelor in analytical chemistry in Odense.

PRIME-XS approach works
Moving from the national to the European level, we touch upon PRIME-XS. The NNF-CPR was an active player. Olsen: “There were four different Joint Research Activities and we were involved in all four of them.” He himself led JRA3 on post-translational modifications. The PRIME-XS experience proved very positive. “It really was a fantastic atmosphere and community to work in. Putting all these people together who used to be competitors, but were now working in a setting of friendly competition turned out really well. Everyone was open-minded and there was no hesitation among the participants to share their latest findings and new protocols. What I particularly liked was the focus on basic research and technology development, but with a clear objective to develop best practices and spread those across the Access Sites, so that they could be translated into robust and usable protocols.” This combination is what makes initiatives like PRIME-XS worthwhile, according to Olsen. “The European research community at large benefits from our work. We developed new methods and made them broadly available and applicable.” Olsen admits there is room for improvement with the involvement of eastern EU countries. “We tried to get them on board, but it didn’t really have a significant effect. We could focus more on our communication towards those research communities.” He strongly supports the idea of a follow-up. “We could do it in a similar fashion, because the PRIME-XS approach worked very well. We managed to find a good balance between technology development and dissemination through the Access Sites.” In spite of the overall positive experiences and everyone’s commitment, without funding, initiatives like this will not fly. “Money is always an essential driver. You need funding for travelling, for organising meetings, for experimentation etc. Right now, we are all lobbying at the EU level through our national contact points for a new call on proteomics or at least on proteomics technology development.”

Like Formula 1 racing
If that funding comes through, Olsen has no trouble in coming up with ideas for new research projects. “So far, cell culture based models are the core of proteomics. A major challenge is to work with patient samples and biopsies. Of course this requires new sample preparation methods, because we need to be able to ‘freeze’ the in vivo state of the proteome and its post-translational modifications. But we also need much more sensitive and faster MS data collection, maybe even a 100-fold increase in both.” No shortage of ambition here, but Olsen puts his statement into perspective. “We basically achieved a 100-fold increase in sensitivity and speed over the past ten years, so we might be able to do that again in the coming ten years.” For Olsen, achieving such a technological step forward would be rewarding in the context of his own research focus. “My main interest is in quantitative proteomics to study cellular signalling pathways in a time-resolved manner. The best proxy for that is phosphorylation, so in my group we have a strong focus on phosphoproteomics. Cell culture models are still dominant, but we are increasingly exploring the possibilities of tissue-based measurements.” Having said that, his fascination for proteomics is also driven by the speed of developments in the field. “The developments in both proteomics technology and applications are still moving very fast. I like to draw the analogy with Formula 1 racing. We tend to think that they cannot go any faster, but then every season there is a new car that out-races the rest.” Olsen doesn’t want to trail behind. “Let’s face it, we all want to race in the fastest machine.”

Career
2014 Professor of mass spectrometry for quantitative proteomics, University of Copenhagen
2012 Vice Director, Novo Nordisk Foundation Centre for Protein Research, Copenhagen
2009 Group leader, Novo Nordisk Foundation Centre for Protein Research, Copenhagen
2006 Post-doc, Max Planck Institute of Biochemistry
2006 PhD, Matthias Mann-lab, University of Southern Denmark / Max Planck Institute of Biochemistry, Martinsried (Germany)
2003 Staff scientist, MDS Proteomics (founded by Matthias Mann)

Key publications
On January 26 and 27, around two hundred proteomics adepts visited the Media Plaza in Utrecht to attend the annual NPC Progress Meeting. A lively crowd of academic and industrial researchers, students and company representatives gathered to share knowledge and skills for the two day event. Participants also took the opportunity to network and establish contacts. The lectures, presentations, discussions and poster sessions were of high quality and the topics were diverse. Here we present an overview of the main trends and developments on show at the Progress Meeting.

The co-organiser of the 10th edition of the NPC Progress Meeting was PRIME-XS, a European consortium of leading proteomics institutes and laboratories. This joint organisation inspired the meeting’s central theme: ‘Proteomics in a European Context.’ Keynote lectures were presented by renowned European as well as Dutch representatives of the proteomics community. The subjects discussed were, with no exceptions, very rewarding.

Continuing a lively, Europe-wide community
The 2015 NPC Progress Meeting clearly demonstrated that great leaps forward are being made in proteomics research. Novel mass spectrometry methods and surprising bioinformatics tools allow us to enlarge our insight into the structure and function of the cellular protein balance. “Our ultimate goal is to unravel the molecular base of life,” according to Albert Heck. “To reach that goal we need to understand cellular biology at the molecular level: each process from DNA to metabolite, including the proteins involved.” Insights and knowledge are being gained step by step. Several interesting pieces of the puzzle were presented during the Progress Meeting and are summarised here. The overview of presentations and lectures is categorised into three areas: analysis techniques, molecular biology applications and bioinformatics. NPC is convinced of the value of this annual proteomics conference. Therefore every effort will be made to acquire support from sponsors and research partners for the 2016 Progress Meeting, for which we have already defined a theme: cancer proteomics. Next year we hope to once again welcome the lively and still growing proteomics community. After all, the Progress Meeting is the place to be for networking and discussing current topics in proteomics research.
Nowadays proteomics and bioinformatics are inextricably tied together. This integration pushes the frontiers in proteomics. Current bioinformatics research reveals well-assorted toolboxes consisting of web-based platforms, algorithms and software packages for genome assembly, annotation, data processing, data mining and sequencing. These tools are helpful for unravelling protein structures and functions, identifying proteins, searching protein databases and profiling protein expression. During the Progress Meeting two experts from the bioinformatics field made their appearance: Lennart Martens (VIB Department of Medical Protein Research, Ghent University, Belgium) and Juergen Cox (Max Planck Institute of Biochemistry, Martinsried, Germany). Their research will be explained in more detail in the next issue of NPC HighLights in the fall of 2015, the theme of which is ‘Proteomics & Bioinformatics.’

Lennart Martens discussed an innovative and promising bioinformatics approach for composing the complete human proteome from public proteomics data: so-called crowd-sourcing proteomes. The counterpart of this approach is extensively investigating the whole proteome simultaneously and publishing it all at once. That is the bazaar versus the cathedral approach, as he respectively labelled the two methods. The bazaar approach has been made possible by the advent of data sharing via public repositories and databases such as PRIDE and the ProteomeXchange initiative. Nevertheless, processing and re-analysis of public proteomics data currently remain far from routine practice. To maximise the value of public proteomics data, reuse and repurposing must become straightforward, allowing the completion of the proteomics data cycle. Martens presented PeptideShaker: proteomics informatics software that can be used at any stage in the proteomics data cycle for the analysis and interpretation of primary data, enabling data sharing and dissemination and re-analysis of publicly available proteomics data. Actually, PeptideShaker provides the gateway to the bazaar. He demonstrated that the cathedral proteomes are complementary to the ones from the bazaar. He furthermore justified that data sets are differently affected by counterfeits, which makes cathedral building difficult. The bazaar can circumvent this issue by relying on the sheer power of numbers.

Juergen Cox presented the usefulness of MaxQuant for analysing large protein data sets in view of protein False Discovery Rates (FDR). MaxQuant is a quantitative proteomics software package designed for analysing high-resolution MS data sets. Cox explained that the control of wrongly identified proteins from such big shotgun proteomics dataset is a nontrivial computational task. He found a way in which this can be done reliably, and his team at the Max Planck Institute implemented it for everyone to use in the MaxQuant software. The team utilised a hierarchy of seven different protein databases, among them SwissProt, TrEMBL and Ensembl, in order to find explanations for the proteins they found in the raw data. These protein identifications range from conventional well-known proteins to novel ones which originate from genomic regions that were previously not known to be encoding the information for building protein sequences. Cox demonstrated the new application by reanalysing shotgun proteomics data from four different studies that had already been published in literature, in particular also including one of the draft human proteomes recently published in the journal Nature. His main finding is that with appropriate control of false discoveries, the number of proteins identified in this draft proteome is not higher than in one of the previously published studies. This demonstrates the importance of applying robust statistical methods in the process of identifying the set of all proteins from shotgun proteomics data. ☺
Analysis Techniques

Mass spectrometry techniques developing rapidly

Present-day proteomics research spans a continuum from qualitative proteomics to quantitative proteomics. Qualitative proteomics implies identification and characterisation of proteins related to basic scientific or specific clinical questions. Quantitative proteomics aims to acquire quantitative information about proteins in order to validate their relevance across larger biological sample sets. Mass spectrometry is the gold standard for both qualitative and quantitative proteomics. The many different methods and techniques enable analysis of complex samples and provide a combination of high sensitivity, mass accuracy and resolution, and high-dynamic range. They also allow researchers to obtain large amounts of data quickly and reproducibly. Speakers presented some appealing examples of technological developments during the Progress Meeting.

Q Exactive HF mass spectrometric instrumentation
Jesper Olsen (NNF Centre for Protein Research, University of Copenhagen, Denmark) presented the advanced Q Exactive HF mass spectrometric instrumentation. This ultra-high-field Orbitrap mass analyser represents the latest generation of orbitrap mass spectrometers. The equipment is still getting smaller and faster and enables ultra-deep analysis of proteomes and post-translational modifications. The instrument is capable of sequencing speeds above 20 Hz and it routinely exceeds 10 peptide spectrum matches per second or up to 600 new peptides sequenced per gradient minute. After combining this MS instrument with high-pH reversed-phase fractionation it proved possible to identify 4400 human proteins in one hour. In addition, very deep proteome coverage was achieved by identifying more than 140,000 unique peptides from 14 high-pH reversed-phase fractionated peptide samples, each analysed by one hour LC-MS gradients. Finally, Olsen illustrated the potential of the technique for answering biological questions related to functional proteomics. His research group applied the advanced MS instrument successfully to decipher the complex nerve growth factor signalling process in neuroblastoma.

A quantitative proteomics tool to identify DNA-protein interactions
Nina Hubner’s research (Institute for Molecular Life Sciences, Radboud UMC Nijmegen, The Netherlands) focuses on DNA-protein interactions. In particular, she studies the interaction between transcription factors and genomic DNA and its impact on disease and cell fate. DNA pull-downs from crude lysates combined with quantitative mass spectrometry provide a powerful method to study protein complexes that bind to certain DNA sequences. This approach however is limited by the required metabolic labelling techniques and the need for large amounts of material. Hubner and her colleagues developed an alternative approach to overcome these limitations. Their efforts resulted in a high-throughput-compatible DNA pull-down that combines on-bead digestion with direct dimethyl labelling or label-free protein quantification. In her lecture, she showed the successful use of this new tool to efficiently identify transcription factors binding to their consensus DNA motifs. Hubner demonstrated the usefulness of the method in GWAS studies. Integrating the proteomics tool in post-GWAS workflows delivers a powerful combination that can provide an unbiased, detailed picture of GWAS loci and their mechanistic involvement in disease.

Proteogenomics workflows
Boris Maček (Proteome Centre Tuebingen, University Tuebingen, Germany) discussed proteogenomics strategies based on high accuracy mass spectrometry to refine the genome annotation of several model organisms. Recent advances in mass spectrometry have led to increased number of applications of shotgun proteomics enabling refinement of genome annotation. Typical proteogenomics workflows rely on the mapping of peptide MS/MS spectra onto databases derived via six-frame translation of the genome sequence. Unfortunately, these databases contain a large proportion of spurious protein sequences which make the statistical confidence of the resulting peptide spectrum matches difficult to assess. Maček’s research group recently performed a comprehensive analysis of the E. coli proteome and mapped the corresponding MS/MS spectra onto a six-frame translation of the E. coli genome. They showed that the posterior error probability distribution of novel hits is almost identical to that of reversed (decoy) hits. They could estimate the sensitivity, specificity, accuracy, and false discovery rate in a typical bacterial proteogenomic dataset. In such a way the use of a small and well-annotated bacterial genome enabled estimation of genome coverage achieved in
state-of-the-art bacterial proteomics. The identified peptide sequences mapped to all expressed *E. coli* proteins but covered 31.7% of the protein-coding genome sequence. These results showed that false discovery rates can be substantially underestimated even in ‘simple’ proteogenomic experiments obtained by means of high-accuracy MS. In his lecture Maček emphasised the necessity of further improvements concerning the coverage of peptide sequences by MS based methods. His group currently pursues this approach to study mistranslation in bacteria.

Mass cytometry

Bernd Bodenmiller (Institute of of Molecular Life Sciences, University of Zuerich, Switzerland) explained the mass cytometry technique and showed how it can fulfil a valuable, complementary role in proteomics research. Mass cytometry is a variant of the classic fluorescence-based flow cytometry. Instead of using antibodies tagged with fluorophores (in which spectral overlap quickly limits the number of parameters available for simultaneous detection), mass cytometry relies on antibodies tagged with transition element isotopes. A special feature of mass cytometry is that it allows single cell studies. By measuring metal abundances, the marker expression can be determined in individual cells. Bodenmiller’s group focuses on CyTOF mass cytometry methods for quantitative analysis of trans-cellular circuits. In his lecture Bodenmiller illustrated the use of their CyTOF method in studying cancer metastasis. The so-called epithelial to mesenchymal transition (EMT) process seems to play a critical role in malignant tumour development. Single-cell mass cytometry provides a suitable technique to map phenotypes of metastatic EMT states. Bodenmiller’s group succeeded in unravelling EMT signalling networks in primary prostate cancer. Finally, he discussed recent results in the field of imaging mass cytometry. Mass cytometry has previously been applied only to cell suspensions. To gain spatial information, the researchers have coupled immunohistochemical and immunocytochemical methods with high-resolution laser ablation to CyTOF mass cytometry. This approach enables the simultaneous imaging of 32 proteins and protein modifications at subcellular resolution; with the availability of additional isotopes, measurement of over 100 markers will be possible. Applying this approach in studying breast cancer proved to allow delineation of cell subpopulation and cell-cell interactions. This contributes to gaining insight into tumour heterogeneity.

Mass analyser based on combined dual fragmentation

Fan Liu (Biomolecular Mass Spectrometry and Proteomics, Utrecht University, The Netherlands) presented a novel integrated workflow to efficiently and reliably identify disulphide-bridged peptides and cross-linked peptides. The system integrates: (1) tailored protein digestion, (2) dual fragmentation and (3) dedicated search algorithms (SlinkS/XlinkX). The dual fragmentation, ‘EThcD’, was recently introduced by Albert Heck’s group and combines electron transfer dissociation (ETD) with higher-energy collision dissociation (HCD). EThcD has been implemented on an Orbitrap MS. The ETD step is applied to fragment the isolated MS precursor, and subsequently all ETD resulting ions are subjected to HCD fragmentation, generating a mixture of b/y and c/z ions. In her lecture Liu explained the importance of increasing our insight into disulphide-bridging and cross-linking. Disulphide bridges are one of the most common post-translational modifications and are essential for the folding, structure and function of proteins, whereas cross-linking plays a major role in protein-protein interactions and protein complexes. Identification of the peptides involved is important for a detailed understanding of protein structures, which directly affect their biological function. She showed the successful use of the newly developed workflow for mapping disulphide bridges in therapeutic antibodies and protein cross-links in ribosomes and proteasomes. ETD preferentially leads to a breakdown of the bridge or the cross-link generating two cleaved peptides as primary fragment ions. Subsequently, HCD primarily targets unreacted and charged precursor ions inducing peptide backbone fragmentation. Finally, detected fragments are analysed by SlinkS/XlinkX which first determine the precursor masses of the two cleaved peptides and subsequently sequence each of them from a linear peptide database.
Mass spectrometry-based proteomics is an indispensable research tool for studying the composition and function of the proteome — the complement of proteins produced by a cell, tissue, or organism at a given time and under specific conditions. Recent technological and computational advances make the emerging field of systems biology accessible and allow the generation of quantitative protein profiles as well as systematic analysis of large numbers of proteins expressed in a cell. One clear conclusion from the Progress Meeting is that proteomics analysis is becoming increasingly important to understand biochemical pathways, to identify biomarkers, to validate drug targets, and to develop tools for disease diagnosis and therapy monitoring.

**Identifying cell-type-specific signalling flows and biomarkers**

Eduard Sabidó (Proteomics Unit, UFP, Barcelona, Spain) discussed a promising approach for pathway modelling to predict cell-type-specific signalling flows. Relating protein concentrations to cell-type-specific responses is one of the remaining challenges for obtaining a quantitative systems level understanding of mammalian signalling. Not all possible signalling routes are used in a given cell type, and different cell types can exhibit very specific responses to the same signal. The aim is to understand these cell-type-specific signalling responses and include them in quantitative predictive mathematical models. Considering that around 230 different human cell types exist, the essential question is whether one indeed needs to quantify all components and signalling flows with all ligands in all cell types. Sabidó presented an effective approach to tackle this problem. Using complementary approaches to identify general and cell-type-specific signalling proteins, his research group succeeded in generating a mathematical model that proved to be suitable for predicting quantitatively protein signal pathways in the complex ErbB network. Next, he discussed another challenging research field within his group. It concerns a search for specific protein biomarkers to diagnose multiple sclerosis. The principle is based on identifying proteins in cerebrospinal fluid samples from multiple sclerosis patients and comparing the results with protein profiles in cerebrospinal fluid samples from healthy people. They succeeded in validating semaphoring 7A and ala-8-his-dipeptidase as biomarkers associated with multiple sclerosis.

**Breast cancer proteomics**

Wilbert Zwart (Department of Molecular Pathology, Netherlands Cancer Institute, Amsterdam) is engaged in research on hormone-associated cancers. In his lecture Zwart examined current research on breast cancer proteomics. These studies lead to better understanding the underlying mechanisms of drug action and drug resistance in metastatic progression of breast cancer, in order to achieve personalized medicine. Breast cancer is the most frequently diagnosed malignancy among women worldwide, with annually around 1.4 million new cases and half a million patients who die from the disease each year. Seventy-five percent of all breast tumours are of the luminal subtype and tumour cell proliferation is thought to depend on the activity of the oestrogen receptor (ER). Inhibition of ER by hormonal therapies is therefore a major treatment modality for these tumours. Unfortunately, resistance to this treatment is often found. In metastatic breast cancer treatment, treatment selection is challenging since biomarkers are lacking. Zwart’s research group tries to unravel the underlying molecular mechanisms of treatment resistance in breast cancer which may lead to the development of biomarkers to guide treatment decision-making. They perform immunoprecipitation of Estrogen Receptor in human tumour specimens, followed by mass spectrometry of the isolated proteins. Using this method, they investigated protein complex formation in tumour specimens, and the changes thereof during tumour progression. They identified specific proteins that may function as biomarkers to identify patients who would likely not respond to hormonal therapies in the metastatic setting.

**Unravelling ubiquitin-regulated signalling networks**

Daniele Guardavaccaro (Developmental Biology and Stem Cell Research, Hubrecht Institute, Utrecht, The Netherlands) introduced the ubiquitin-proteasome system and its important regulatory role in fundamental cellular functions such as DNA replication, DNA repair, transcription, protein synthesis, cell differentiation and apoptosis. Ubiquitin is a small protein that is covalently attached to protein substrates, generally targeting them for degradation by the proteasome. The precision and
selectivity of the protein degradation machinery is conferred by the diversity as well as the specificity of ubiquitin ligases. The identification of the many different substrates of ubiquitin ligases is crucial to understand the molecular mechanisms controlling key cellular processes. With this goal, the Guardavaccaro and Heck laboratories developed a novel approach based on affinity purification followed by mass spectrometry. Guardavaccaro illustrated this successful approach, which led to the discovery of new molecular networks controlling epithelial cell migration whose defective regulation is implicated in pathological processes such as fibrosis and cancer metastasis. The researchers found that metastatic factors trigger the ubiquitin-mediated degradation of RAPGEF2, an activator of the small GTPase RAP, which in turn controls cell adhesion, migration and polarity by regulating the activation of integrins. Remarkably, failure to degrade RAPGEF2 inhibits invasion and metastasis of human breast cancer cells. Ultimately, this study indicates that by blocking epithelial cell motility and invasion, degradation-resistant forms of RAPGEF2 might yield beneficial effects against the metastatic dissemination of cancer cells and may result in the exciting possibility of modulating the activity of specific ubiquitin ligases as a way of developing mechanism-based anticancer therapeutics.

PlantProteomics: challenged by the chloroplast factory

Myriam Ferro’s research (CEA, iRTSV, Laboratoire Biologie a Grande Echelle, Grenoble, France) is focused on subcellular protein localisation, especially in plants. Subcellular localisation is an important feature for understanding protein function and elucidating interactions in the cellular machinery. This field of ‘subcellular protein localisation’ is called organelle proteomics or spatial proteomics. Present mass spectrometry methods allow localisation of many proteins in a single set of experiments. Ferro presented recent research on deciphering the protein distribution within the chloroplast. This organelle has a number of essential functions, including photosynthesis and synthesis of amino acids, fatty acids and many secondary metabolites. Chloroplasts contain several sub-compartments: i) the chloroplast envelope, which is a double membrane system surrounding the organelle; ii) the stroma, mainly composed of soluble proteins, which is the site where CO₂ assimilation takes place via the Calvin cycle; iii) the thylakoid membrane, which is a highly organised internal membrane network and the centre of photosynthesis. Using special bioinformatics toolkits (pRoloc and Proline²), Ferro’s research group succeeded to map the whole chloroplast proteome of the model plant Arabidopsis thaliana. Recently the researchers went a step further in the geolocalisation by investigating the sub-chloroplastic thylakoid compartment. About 300 thylakoid (or potentially thylakoid) proteins appeared to be enriched. Except that their findings corroborated previous observations obtained for photosynthetic proteins, new molecular actors for photosynthesis-linked activities have been discovered. This allows generating hypotheses about the assembly/function of thylakoid proteins.

1. pRoloc: developed by the Cambridge Centre for Proteomics (UK), Prime-XS partner.
2. Proline: developed together with partners of the Proteomics French Infrastructure (ProFi; http://www.profiproteomics.fr/).

Understanding derailed metabolic pathways in diseases

Celia Berkers (Biomolecular Mass Spectrometry and Proteomics, Utrecht University, Utrecht, The Netherlands) leads a Metabolomics group. Metabolic pathways and metabolite levels in cells change as a result of gene and protein activities to meet the cell’s physiological demands. Because most diseases and the action of drugs also induce metabolic changes, studying the metabolism of diseased or drug-treated cells can aid in identifying new targets for therapeutic intervention. That is exactly the research area of the Utrecht Metabolomics group, which uses mass spectrometry techniques to investigate metabolites and metabolomics pathways. One of Berkers’ research lines aims at unraveling the response of cells to proteasome inhibitors — drugs that block the proteasome, the main protein degrading machinery in the cell. A well-known proteasome inhibitor is the anticancer drug bortezomib. Although this drug is now used in the clinic for the treatment of cancer, treatment is also often associated with the occurrence of drug resistance. In her lecture Berkers showed that metabolic changes contribute to bortezomib resistance. Metabolomics studies demonstrated that bortezomib-resistant cells are forced to use an unusually large part of their glucose uptake for anti-oxidant purposes. Because glucose supply is limited, this resulted in metabolic stress on other pathways that also depend on glucose and forced cells to increase uptake of specific nutrients from the growth medium. Berkers showed that such metabolic stress can be exploited; starving the cells of these nutrients re-sensitised bortezomib-resistant cells to the drug. These findings indicate a potential role for nutrient starvation in the treatment of bortezomib resistant tumours. 🍃
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The Young Ones

Wow! Imagine being a real university student for a day. It proved a great experience for the group of primary school pupils that visited the Heck lab. They were introduced to the secret world of proteins. The children, aged 10-11, attended lectures and performed experiments in the laboratory. For these future scientists, the university day was a real winner.

The pupils attend the so-called Day a Week School (DWS) in Zeist, a small town close to Utrecht. The DWS is geared to highly talented primary school pupils, who spend four days a week at their regular school and one day at DWS. These boys and girls come from different schools, but share a need for additional challenges. To keep up their enthusiasm and motivation, these children need to be addressed at their own cognitive level. Spending a day at Professor Albert Heck’s Biomolecular Mass Spectrometry and Proteomics group proved a great way to expand the horizons of these bright young minds.

Theory and practice
The day started with ‘lectures’ just like any normal day at college would. The ‘students’ learned about proteins from Albert Heck himself and from Reinout Rajmakers. The teachers explained what proteins are and their role in our body, and they explained the DNA that contains the genetic code for protein production and function. During a tour of the laboratories, the junior students were introduced to the mass spectrometric methods for analysing these proteins. Then, of course, the children got to work themselves. PhD student Renske Penning showed them the ropes for doing real laboratory experiments. They carried out colour tests to determine the abundance of proteins in lemonade. They also analysed milk, soft drinks and apple juice and compared the outcomes with their analysis of pure water. All in all, as shown in the pictures, the children had a fun and valuable experience. The scientists in turn, got a glimpse of the promising next generation that may harbour their successors. A win-win for both sides, hosts and guests.

Future scientists Catch them while they’re young, as the saying goes, and that certainly is true for programmes such as the one-day-university initiative as well.
Et in Arcadia Ego

Art and science team up to confront cancer

Art that uses living material and scientific processes, that is bio art, offers new perspectives on science. The work of artist Charlotte Jarvis is a profound example of this. Her latest project is about attempting to grow her own tumour outside of her body. This new bio art creation, entitled Et In Arcadia Ego, is based on close collaboration with Hans Clevers’ research group on Stem Cells and Intestinal Cancer at the Hubrecht Institute in Utrecht.

Charlotte Jarvis was artist-in-residence at the NPC for over two years, during which time she created Blighted By Kenning (which involved encoding the Universal Declaration of Human Rights into DNA & spraying it onto the surface of ‘apples of knowledge’) and Ergo Sum (a ‘second self’ of beating heart cells, firing neurons & flowing blood vessels created from stem cells). As a partner project to the stem cell doppelganger Charlotte created for Ergo Sum, she is now working to grow her own biological ‘nemesis’ in the form of a malignant colon tumour. This will be cultivated in vitro from her bowel cells. Once the cancer has been grown, Charlotte plans to ‘perform’ with it in a gallery space to explore mortality and investigate how we think about cancer and the role it plays in our lives.

Stimulating discussion

It seems counter-intuitive that someone would want to grow their own cancer, but Jarvis sees it as helpful for stimulating discussion about the nature of the disease: “People often think about cancer as an alien invader, transgressing our bodies, but it’s a constantly evolving part of us, which is why it’s so difficult to treat.” She wants to further the exploration of biological identity that she began in her earlier work, Ergo Sum: “Modern technology has muddied the boundaries between what is and isn’t my body. If I can grow my heart cells outside my body, are they part of me? And is a tumour grown externally any less ‘me’ than those heart cells?” Professor Hans Clevers and his colleague Jarno Drost have developed a method for simulating the mechanism by which colon cancer occurs. They are collaborating on the project to grow Charlotte’s cancer, and will retain some of her collected cells for use in their research. Currently all of the samples available to them are taken from patients being treated for cancer (even though they are taken from non-cancerous sites). This will be the first time that they will have non-patient cells for research.

The challenges

Bio-art projects, by their adventurous and experimental nature, are notoriously prone to hurdles. We asked Charlotte what she has struggled with. “The biggest difficulty has been working with medical ethics. It has been very difficult to find an institute willing to take a colon biopsy [a relatively minor procedure], as it has no medical benefit and carries a tiny risk. I find this frustrating, given that many of the clinics which we have approached regularly carry out extensive cosmetic surgery, which is comparatively high risk and arguably has no medical application.” Charlotte thinks she has now found a doctor willing to give her the rectoscopy necessary to gather bowel cells, but is also in talks with a scientist in the US who, as an alternative, could grow colon tissue from her stem cells already grown for Ergo Sum. If Charlotte decides to employ the latter method, it will represent a new avenue not yet explored in cancer research. After this, the next stage will be to actually grow the cancer, and Charlotte aims to display it the completed piece at the end of 2015 or early 2016.

The tumour will be gut cancer that is genetically ‘Charlotte’ — grown from her own cells in the lab. The tumour will eventually live in a specially designed incubator suitable for gallery spaces. But that is not the only reason why this project is remarkable. The sample will also be used in professor Hans Clevers’ scientific research. He uses stem cells to grow samples of colon tissue in vitro. To this date all of the samples he has had access to are from cancer patients. This project would provide him with the first opportunity to test his method on tissue from a healthy patient sample.

Images from http://www.artforeating.co.uk/ © Charlotte Jarvis.
Top Publication

“Our data reveal the reprogramming process itself.”

Current protocols for reprogramming adult cells to stem cells are time consuming and highly inefficient, Muñoz explains. “The process takes almost three weeks and the yield is roughly 1 percent. So, the thought was, if we understand the process, we may be able to optimise it.” This was the starting point in 2010 for Project Grandiose, a large international consortium initiated by Andras Nagy of the Lunenfeld-Tanenbaum Research Institute in Toronto who had developed a new reprogramming protocol with a considerably higher yield. Muñoz explains: “The Nagy-group pulled samples every 2-3 days and ‘froze’ the process there. This means we all had the same samples taken at fifteen time points. Not only start and finish, but thirteen moments in between as well. Most similar studies use far lower numbers of samples and they don’t all work with exactly the same material. We did so, which is why we could really put the data from the different molecular levels together to create one overall picture.”

Dynamic behaviour
The results supported findings from earlier studies that the proteome of the differentiated cell is very different from the iPS proteome. That was to be expected, but much more interesting is that this endeavour revealed the highly dynamic behaviour of protein expression levels during reprogramming. Muñoz: “Many expression levels were going from on to off and vice versa. If you only measure the differentiated and the iPS proteomes, you don’t see these huge changes. Our data reveal the process itself.” And there was much to see. For example, after two days the expression levels of 40-50% of the proteins showed a difference. To Muñoz’ surprise: “I didn’t expect an effect on the protein level that quickly. We thought that changes in protein levels might be delayed. First changes on the lower regulatory levels have to take effect and then we will see changes on the protein levels, but that is not the case.” Another remarkable event occurred between day 16 and day 18. Or better: the surprising thing was that almost nothing happened. “But something was definitely going on somewhere else, because after day 18 we saw again a lot of changes in the proteome.” What stirred up the action again? That is still a matter for further investigation.

Project Grandiose

Proteome changes during cellular reprogramming

The discovery of the induced pluripotent stem cells (iPS) is one of the major breakthroughs in biology in recent years, says Javier Muñoz, head of the Proteomics Core Unit at the Spanish National Cancer Research Centre (CNIO) in Madrid. “We can now reprogram adult cells and turn them ‘back’ into pluripotent cells, but we don’t really understand how the reprogramming process works”. An international team of researchers cracked the molecular code. In December 2014 the extensive results appeared at the same time in five publications: two in Nature and three in Nature Communications.

Sounds straightforward, but it was a major challenge, Muñoz says. “It is extremely difficult to solve a puzzle if you have to simultaneously solve different, but interconnected puzzles first. Also the pieces have different formats, making the whole process even harder.” But in the end, they managed resulting in not just one, but also a set of five top publications1.

Javier Muñoz (l) and Albert Heck. The Heck lab team supported the project Grandiose with their proteomics expertise. Javier Muñoz, one of the protein specialists, explains the role and impact of the proteomics part.

1. Five papers: see www.stemformatics.org

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European Summer School Advanced Proteomics 2015

Mass spectrometry in the mountains

The European Summer School Advanced Proteomics has established itself as one of the premier occasions to learn everything you need to know about proteomics, meet fellow students and interact with the leading proteomics experts in an informal setting.

From August 2-8, 2015, the 9th edition of the European Summer School Advanced Proteomics will take place at the traditional location of the ‘Kloster Neustift’ monastery in the Italian Alps. Simone Lemeer, Assistant Professor at the Biomolecular Mass Spectrometry and Proteomics group, Utrecht University is on the organising committee. “Our primary target audience are PhD students and postdocs who are relatively new to the proteomics field and want to learn the basic concepts,” says Lemeer. “Participants are selected based on the abstract they submit and each year we have more candidates than we can accommodate.” The summer school takes one week and the programme covers basic lectures on the theory, hands-on workshops and presentations on current research. In addition, all participants have to present a poster and give a 1-minute pitch on their research. “What is especially nice about this summer school is that most speakers stay for the whole week, so there is ample time for the participants to talk to them. On 70 participants, we have approximately 25 speakers present, which is quite extraordinary.”

Social activities
Besides the science, there is also room for social activities, such as hiking in the beautiful mountains that surround the monastery, rafting, swimming and of course just relaxing with a drink during the evenings. “It is a very intensive programme,” Lemeer admits. “On Friday, everyone is pretty exhausted, but so far the participants have always been very positive in their feedback. The combination of high level presentations and discussions, combined with the really easygoing atmosphere and the opportunity to talk to leading scientists in the field is very much appreciated.”

Great opportunity
One the participants who agree on that is Anna Ressa. She is a PhD student in the group of Albert Heck in Utrecht and participated in the 2014 edition of the summer school. Why did she sign up? “I started my PhD research in January 2014 and I was new to both proteomics and mass spectrometry. I thought that after a few months of training in the lab, this summer school would be a great opportunity to merge the lab experience with the theory and learn about different approaches,” Ressa explains. She was not disappointed. “I never expected a summer school could be organised in such a good way, balancing the time between education, activities and networking with the other students and scientists. I particularly valued meeting so many people, fellow students from other labs and all from different countries as well as the most famous scientists in proteomics. It was really nice to interact with people that all share a passion for science.” Not surprisingly, Ressa highly recommends this summer school to others. “If you want to be part of the proteomics world, this is a unique opportunity to learn from the experts.”

Sign up for edition 2015
European Summer School Advanced Proteomics
August 2-8, 2015
Kloster Neustift, Brixen/ Bressanone, South Tirol, Italy
www.proteomic-basics.eu
Deadline for registration and abstract submission: 15 May 2015
Valorisation for scientists

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NPC Progress Meeting

In attracting around 200 attendees and having inspiring lectures of internationally leading scientists, this year’s edition of the NPC Progress Meeting proved again to be a success. Furthermore, young scientists were given the opportunity to feature their research during the NPC poster session. We congratulate the following researchers with their Poster Prize:

1. Elisabeth Stes: UGent — VIB, Belgium
2. Jens Milbradt: Universität Erlangen-Nürnberg, Germany
3. Andrea Brunner: Utrecht University, the Netherlands.

An impression of both days is available on: www.netherlandsproteomicscentre.nl

Visit ICI online

The Institute for Chemical Immunology (ICI) is a Graviation project of the Dutch Government, coordinated by Sjaak Neefjes (former NPC theme leader). It will define and exploit a new field termed chemical immunology and aims to train a novel generation of interdisciplinary scientists. Its goal is to promote academic excellence; with economic and clinical translation of new treatment options for a large population of patients that currently only have limited treatment options.

To find out more about ICI, please take a look at the ICI website: www.chemicalimmunology.nl

Facility for Proteomics Research at UMC

As part of the Proteins At Work programme, the UMC recently started with the Facility for Proteomic Research. The facility aims to provide state-of-the-art technology, equipment and expertise to support fundamental, translational and clinical proteomics research. For analysis of complex samples limited bioinformatics support will be provided. “Our latest acquisition, a Thermo Fisher Fusion Tribid LC-MS, is up and running now. Its extremely high resolution and speed will enable us to improve our services,” says Facility Coordinator Dr HarmJan Vos. The Facility will closely collaborate with the NPC and proteomics group at the UU headed by Prof. Albert Heck. Access is available for all researchers from participating institutes (UMC Utrecht, Hubrecht Institute, Utrecht University and Centre for Personalized Cancer Treatment).

For more information please contact HarmJan Vos: h.r.vos@umcutrecht.nl

Upcoming Events

2-8 August, 2015

9th European Summer School Advanced Proteomics Brixen/Bressanone, Italy
This summer school is designed to provide graduate students and young postdoctoral scientists from academia and industry with insights into state-of-the-art proteomic technologies and applications in the life sciences.
www.proteomic-basics.eu

5-8 September, 2015

The EMBO Meeting UK-Birmingham
The EMBO Meeting offers a European forum for scientific exchange. Topics cover the entire range of life sciences, encouraging participants to look beyond the boundaries of their own fields. The scientific programme is supplemented by networking and career development opportunities.
www.embo.org

27-30 September, 2015

HUPO 14th Annual World Congress (HUPO 2015) Vancouver, Canada
Hosted by the Canadian National Proteomics Network (CNPN) in coordination with the BC Proteomics Network (BCPN).
www.hupo.org

9 November, 2015

1st Science for Life Conference Utrecht, the Netherlands
Science for Life is a unique combination of scientists from four of Utrecht University’s institutes that join their fundamental and strategic research capacities in the Life Sciences domain. The first Science for Life conference will take place in November 2015.
http://www.uu.nl/en/research/science-for-life
Proteomics in a European context

Keep up this well-run proteomics machinery

I was lucky enough to attend the NPC Progress Meeting 2015 at the Media Plaza in Utrecht. Reflecting on the superb keynote lectures, the lively corridor chats and the abundance of interesting posters, I could only conclude that proteomics is firmly rooted within the European boundaries. Research groups and institutes have each built their own specific expertise, resulting in an impressive array of centres of excellence in various countries like The Netherlands, Denmark, UK, Germany and Switzerland. These efforts have been spearheaded by renowned scientists: Albert Heck, Matthias Mann, Peter Roepstorff, Ruedi Aebersold, to name just a few.

PRIME-XS fulfilled a prominent role in the successful development of a European proteomics infrastructure and community. The project, funded by the European 7th Framework Programme, enabled intensive cooperation, and has proven to be highly profitable. From my position as a member of the scientific advisory board, I closely witnessed the power of this approach. Besides at least 150 peer-reviewed publications, PRIME-XS generated more than 100 user projects with the participants coming from more than 20 different European countries. By providing their state-of-the-art MS technology, as well as their high level knowledge and expertise, the European proteomics community is actively contributing to biological and biomedical research at the highest level.

As you can read in the report of the Progress Meeting (see p 6-11), very significant headway has been made in mass spectrometry technology and its application in a variety of fields, ranging from the unravelling of cellular processes in plants and tumours to discovering novel biomarkers and lead compounds for pharmaceutical development. It is with a touch of envy that I watch the European approach to networking and collaboration. From an American point of view, it is almost magical, since we tend to operate more independently. I will certainly try to translate my experience with PRIME-XS to the American model, as it has clearly demonstrated that it has provided a superb proteomics infrastructure that in turn generates high quality research and breakthrough results.

In short, you can all be proud of the world-leading European proteomics community. Therefore, I strongly advise you to keep the community lively and active, in spite of the end of PRIME-XS. There is still much work to be done. I am convinced that mass spectrometry-based proteomics will become at least a thousand times faster and more sensitive! Furthermore, we are still faced with a plethora of challenges if we are to unravel the detailed biology of life at the molecular level.

If you can continue the current collaborative approach, I foresee a golden future for proteomics research in a European context. So I hope to participate again next year in the NPC Progress Meeting and to share in the latest successes and developments achieved by the proteomics community.

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