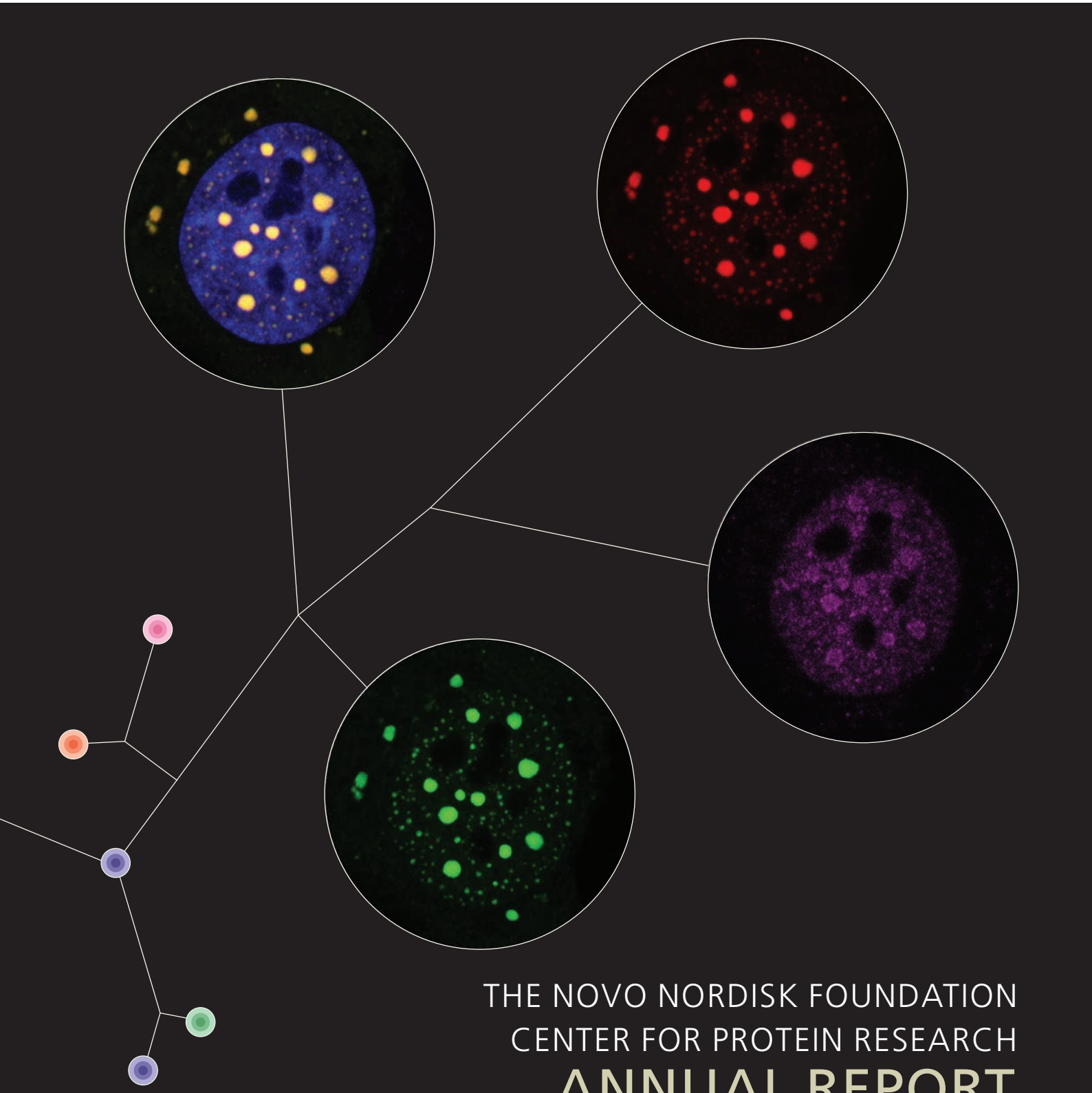
The Novo Nordisk Foundation
Center for Protein Research

UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES



THE NOVO NORDISK FOUNDATION CENTER FOR PROTEIN RESEARCH
ANNUAL REPORT 2015

THE NOVO NORDISK FOUNDATION CENTER FOR PROTEIN RESEARCH

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Front cover shows fluorescence microscopy images of proteins with prion-like features forming liquid droplets inside human cells (from Altmeyer *et al*., *Nat Commun*, 2015).

CONTENTS

FROM THE MANAGEMENT

Jiri Lukas, Executive Director

This has been a year of truly significant developments in the history of The Novo Nordisk Foundation Center for Protein Research (CPR) in terms of contributions made to protein research and the consolidation of both our team of stellar scientists and support initiatives (see Figure 1 for selected milestones during 2015) and last, but not least, our success in attracting external funds for our research – in 2015 this amounted to 150 million DKK (20 million EUR).

Reinforcing CPR's staff

From the perspective of the CPR management, this year's exciting developments in reinforcing our staff of top-tier researchers have had huge significance for the concept of CPR as a whole, validating the strong, innovative research direction and future potential of CPR. In 2015, having coordinated the core proteomics and systems biology activities at CPR for several years, both Matthias Mann and Søren Brunak committed their research careers more closely to CPR.

Matthias received a hugely prestigious Novo Nordisk Foundation (NNF) Award of 60 million DKK (8 million EUR) to launch a new research group dedicated to Clinical Proteomics, which will be integrated into the Proteomics Program, where Matthias already serves as Director.

Søren has relocated from the Technical University of Denmark to CPR, where he now works full time as the Director of the Disease Systems Biology Program and Group Leader of the Translational Disease Systems Biology group.

Proteomics for disease diagnostics

The coordination of Matthias's and Søren's decisions is also excellent, as the new Clinical Proteomics project led by Matthias will strongly benefit from the Big Data Management Platform provided by Søren's program. When combined with the unique, population-wide Danish electronic patient records system and our access to a large and well-curated source of clinical material, we will have hitherto unprecedented power to demonstrate that mass spectrometry has matured to a stage where it can diagnose diseases from protein profiling of a drop of

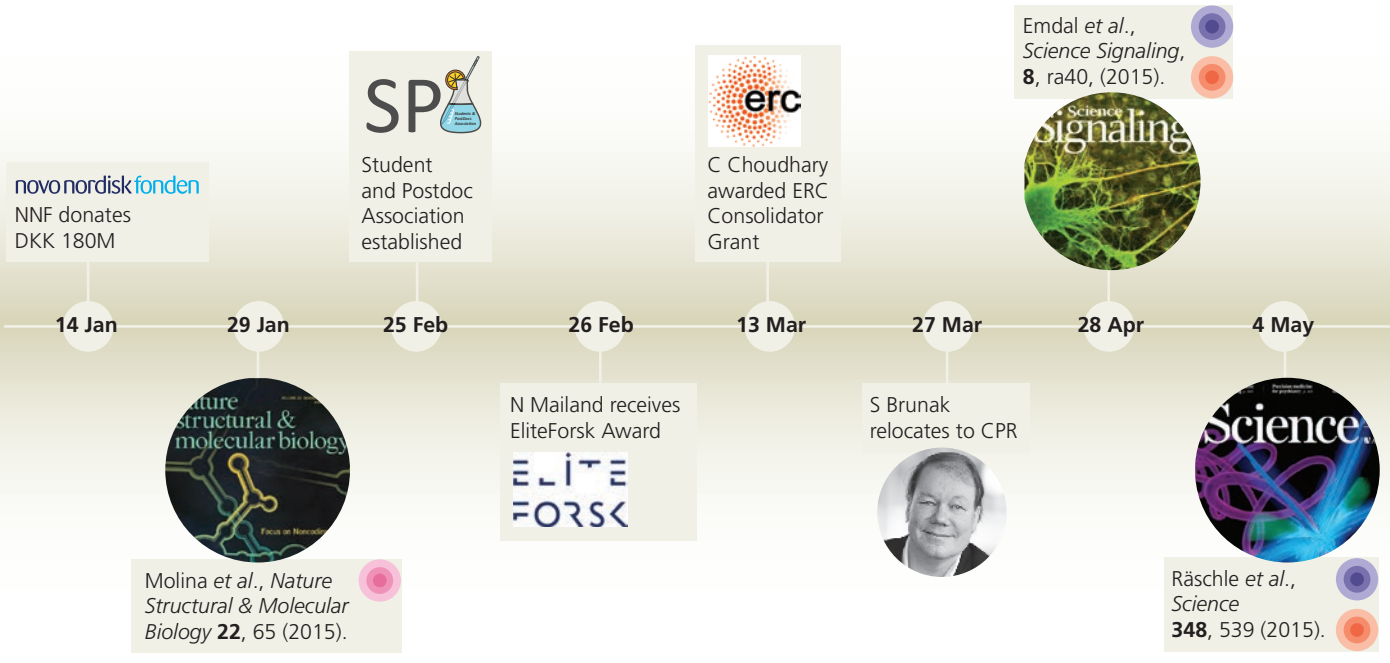


Figure 1 | Selected 2015 key milestones and achievements.

blood from a finger prick.

We are very excited about this development because of its potentially huge scientific and societal impact, and because it further strengthens the CPR vision to synergize blue-sky thinking in basic science with dedicated translational research performed by expert CPR scientists at the highest international level. To reach this point, we have enjoyed exceptional support from the University of Copenhagen's leadership, which is best illustrated by the quote from Ulla Wewer, the Dean of the Faculty of Health and Medical Sciences, on the announcement of Matthias Mann's new grant:

"It is an honor that the University of Copenhagen can manifest itself among the international elite of front line protein research with a world-leading scientist such as Professor Matthias Mann. Translation of basic research to the patient's benefit is among our major missions and we could not have wished for a stronger initiative to achieve this goal."

Recognition of outstanding contributions

While writing this Executive Summary, news came in that Matthias Mann has also been recognized for his outstanding contributions by Her Majesty the Queen of Denmark. Matthias has been awarded the 'The Order of Dannebrog,' a recognition reserved only for those who have made major contributions to Danish Society. We could not have wished for a better and more encouraging recognition of the science produced at CPR. Well done Matthias!

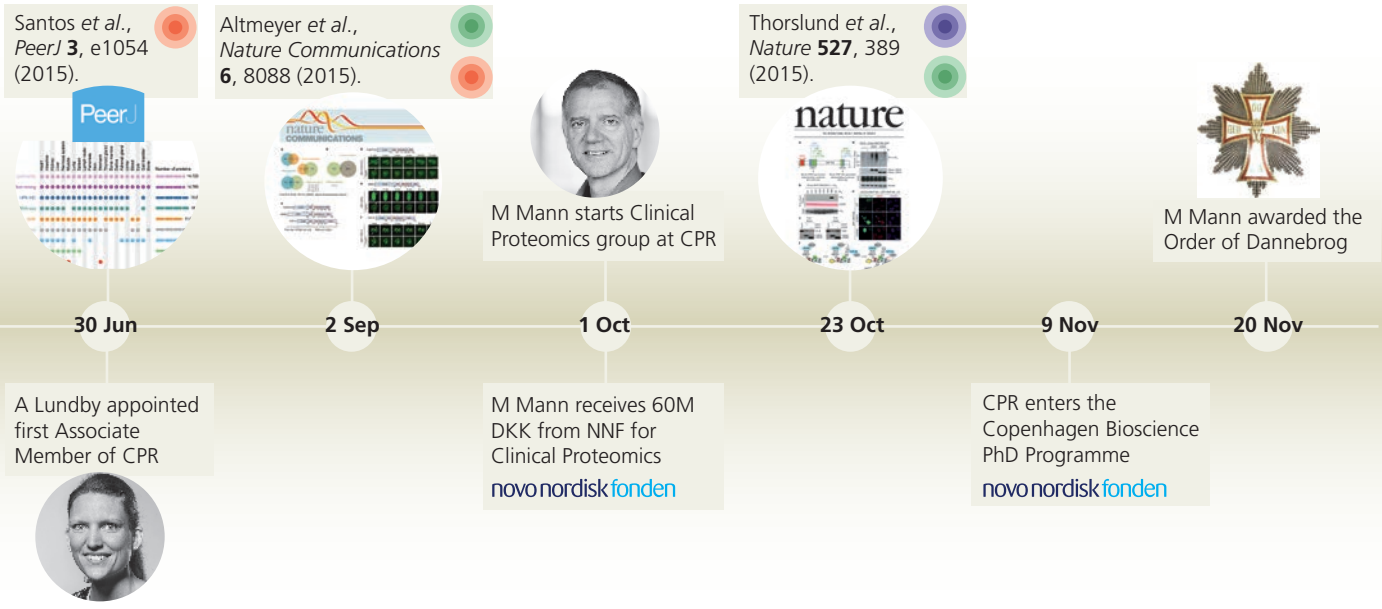
CPR Associate Membership initiative

A key initiative launched in 2015, to support our scientists by strengthening career development and providing unique opportunities to 'home-grown' talent, is a new affiliation called 'CPR Associate Membership.' The primary aim of this affiliation is to help our most successful postdocs to start independent junior groups within the Danish academic environment. These CPR Associate Members will continue to have privileged access to our technological platforms and the opportunity to participate in joint research projects, and will continue to attend CPR meetings and events to increase the possibility of networking with CPR scientists.

We are happy to announce that Alicia Lundby, a former postdoc in the Olsen group and now a group leader at the Faculty's Biomedical Institute (BMI), has become the first CPR Associate Member.

SPA flourishes

Of benefit for all young CPR researchers, we are extremely pleased that the Student and Postdoc Association (SPA), launched in 2014, has become a lasting initiative with a direct and positive impact on the decisions made by CPR management. SPA representatives regularly attend monthly management meetings, have been very successful in inviting and hosting world leaders in protein science, and organized



SPA's first off-site retreat, which was reported to be a huge success.

In return, CPR organized a 2-day course on scientific writing for SPA members, led by Dan Csontos (Elevate Scientific), a top professional in this area and a former *Nature* editor.

Simply put, it is no longer possible to imagine CPR without SPA, a view that is not only our internal perception but one also held by our Scientific Advisory Board (SAB). Tony Hyman, the Chair of the SAB, wrote in his report of their annual visit in 2015:

"The whole SPA concept is very impressive and the organization is a strategic asset to CPR that should be nurtured and championed."

Scientific output

The main aspect of the achievements of 2015 is the remarkable scientific productivity of CPR. Some of the highest impact discoveries include: characterizing the most complete proteome of a defined DNA repair pathway to date; discovering a new facet of the histone code by connecting linker histone ubiquitylation to the ever-growing genome surveillance signaling network; providing unprecedented mechanistic and structural insight into the catalysis of enzymatic hydrolysis of DNA phosphodiester bonds; uncovering the unexpected role of the unstructured human proteome in genome integrity maintenance; generating breakthrough insights into the role of major protein modifications at the systems level; unraveling cellular decision-making and pathogenesis in brain and heart diseases; and developing unique and interactive web resources. Scientists at CPR continue to publish outstanding results in high-impact journals and present their research in effective and engaging ways. A remarkable 24 out of a total 80 scientific papers with CPR affiliations were published in the most prestigious journals, including *Nature*, *Science*, *Annual Review of Biochemistry* and their sister journals.

An important achievement with a strong potential to transform CPR research into a significant societal impact was made by Søren Brunak, who has become a co-coordinator of a white paper describing the large-scale opportunities for precision medicine in Denmark. There have been several other areas in which CPR research has made significant impacts during the last year, and this has strengthened CPR's position as a highly integrated scientific environment where researchers at all levels benefit from mutual synergy.

Funding secured in 2015

Our scientists succeeded in securing funding from many sources in 2015; a 5.7 million DKK (0.8 million EUR) Horizon 2020 project, EU-ToxRisk grant was secured by the Brunak group (Disease Systems Biology Program). The Montoya Group received 9.8 million DKK (1.3 million EUR) from the prestigious NNF Challenge Programme for a collaborative project with the group of Thue Swartz, also from the University of Copenhagen. Chunaram Choudhary (Choudhary group, Proteomics Program) received a European Research Council (ERC) consolidator grant to investigate deubiquitylase-regulated signaling in human cells and, last but not least, Luis Toledo from the Lukas group (Protein Signaling Program) received an ERC Starting Grant of 11.3 million DKK (1.5 million EUR).

We look back on our achievements in 2015 with a sense of pride and gratitude, and look forward to building on our success and pushing the boundaries of protein science in the years to come.

Peter Dyrsting
Head of Administration and Finance

Jesper V. Olsen
Vice Director

Jiri Lukas
Executive Director

The Novo Nordisk Foundation
Center for Protein Research

novo nordisk fonden

FROM THE DEAN
ULLA WEWER

Ulla Wewer,
Dean of the Faculty of
Health and
Medical Sciences,
University of Copenhagen

The Novo Nordisk Center for Protein Research (CPR) at the University of Copenhagen (UCPH) Faculty of Health and Medical Sciences has very effectively enhanced the comprehensive 'ecosystem' of health and medical sciences research and education at the University. I am very impressed and proud of the entire CPR team, for their many achievements and advances made in protein technologies and research. With its groundbreaking research results and impressive publications, together with the establishment of new research fields and new partners, CPR is a beacon of inspiration to us all. CPR's research has already demonstrated great medical relevance and it is now making investments in translational research and increased collaboration with hospitals, municipalities, and regions. This will undoubtedly benefit patients both in Denmark and across the world.

It is evident that CPR has cemented its leading position among the top players in the protein research area. CPR has also succeeded in recruiting and retaining top international researchers, as well as developing junior 'rising star' researchers working on innovative projects.

Basic research is the foundation on which education at UCPH is built. CPR is firmly rooted in UCPH and contributes to strengthening basic research, applied research, and education at the University, as well as helping to reinforce Copenhagen's position as an international city of knowledge.

UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

INTRODUCTION TO CPR

Protein-related technologies promise to be even more revolutionary than genomic approaches for furthering our understanding of the complex wiring of biological systems and disease processes, but a concerted effort is required to realize this promise. So far, protein research across the world has been scattered and often performed in isolated units that have not integrated the enormous advances in protein technologies.

This approach has limited the progress in addressing major unmet needs in biomedicine and led to a paucity of highly skilled protein researchers with broad experience of the field. Furthermore, although many countries have made large investments in protein-based technologies, few have assembled all of the required technologies under the same roof at the highest level of excellence, and none have done so in the context of clearly defined biomedical focus areas. CPR was created with the generous support from the Novo Nordisk Foundation (NNF) and the Faculty of Medical Science and Health at the University of Copenhagen (UCPH) to specifically address these issues and integrate and bring together key protein-based technologies and excellent scientific expertise, from several key biomedical fields, under one roof.

MAJOR CHALLENGES IN PROTEIN SCIENCE

Tackling the characterization of complex protein machines, their modifications and interactions, and applying this fundamental biological knowledge to elucidate disease mechanisms is a major challenge facing protein researchers today. The IT and Big Data revolution is reshaping the way we conduct biological research and how we apply this knowledge to biotechnology and healthcare.

Keeping up with this transformation is a formidable challenge for all biologists, including protein scientists. Insufficient knowledge management capabilities for the analysis of high-throughput data are a major bottleneck that is hindering its use in basic and translational research.

Technological developments are also needed to enable omics analysis of body fluids, to work towards its application in the clinic, and to make protein-based precision medicine a realistic prospect. Diseases almost always manifest at the level of proteins. Consequently, drugs are typically directed against proteins or are proteins themselves.

Taking into consideration all these needs and developments, the key unanswered questions in protein science, where we feel CPR can make a real difference, are how metabolic and environmental stresses overwhelm the large number of proteins wired in complex protein pathways that normally protect the genome's integrity and thereby cause disease.

CPR THEN AND NOW

To fill the void in protein research globally, CPR was founded in 2007, with the vision

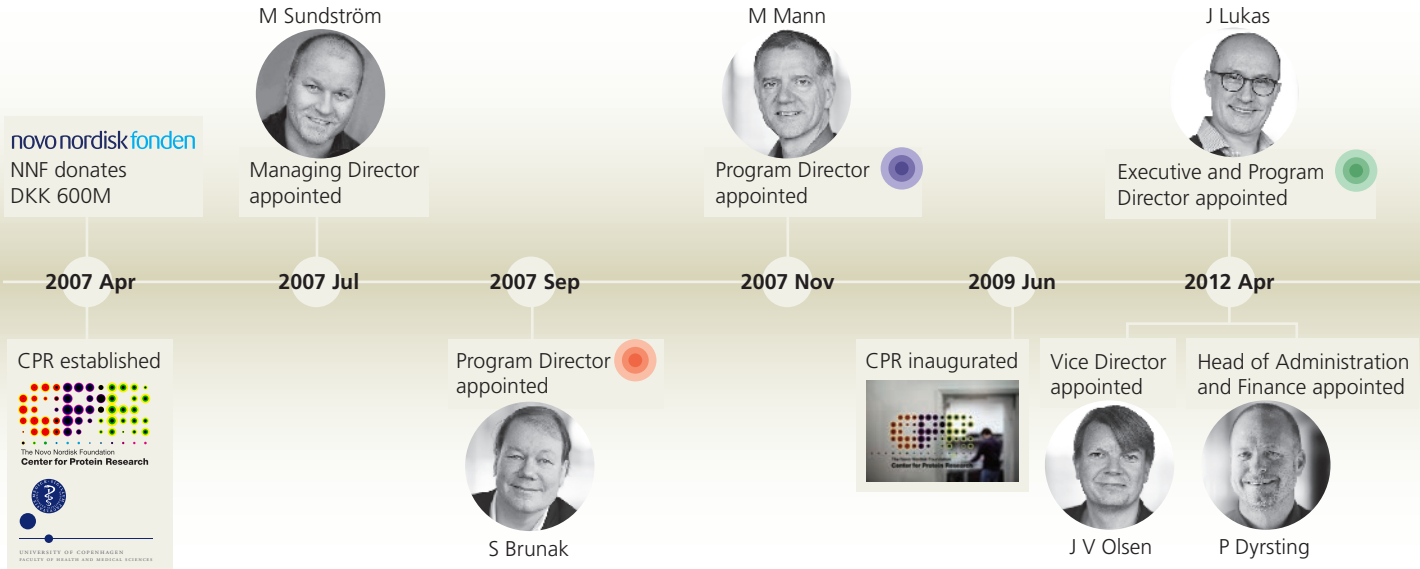


Figure 2 | Selected milestones during 2007-2014.

of becoming a world-leading center in integrative protein technologies and their application to accelerate understanding of the biological processes underlying health and disease. Thanks to the generous support of the NNF and the UCPH, CPR has rapidly established itself on the national and international protein science scene (see Figure 2 for a brief history of CPR). After a very positive scientific review of CPR in 2014, CPR was awarded 180 million DKK (24 million EUR) in extended funding by the NNF for 2015–2019.

From its inception, CPR scientists have used world-class protein technologies and know-how to address major open questions in protein science related to:

- Functional understanding of protein pathways subverted in disease
- Structural characterization of protein machines
- Mass spectrometry-based proteomics
- Functional implications of proteome- and genome-wide data

VISION

To be the world leading center in integrative protein technologies and their application to accelerate understanding of the biological processes underlying health and disease.

MISSION

To develop integrated protein technology platforms and large heterogeneous data management systems to:

1) **Further the understanding of complex protein networks in fundamental biology and disease**
2) **Produce the next generation of top-tier protein scientists**
3) **Become an unmatched global partner in protein research**

THE CPR APPROACH

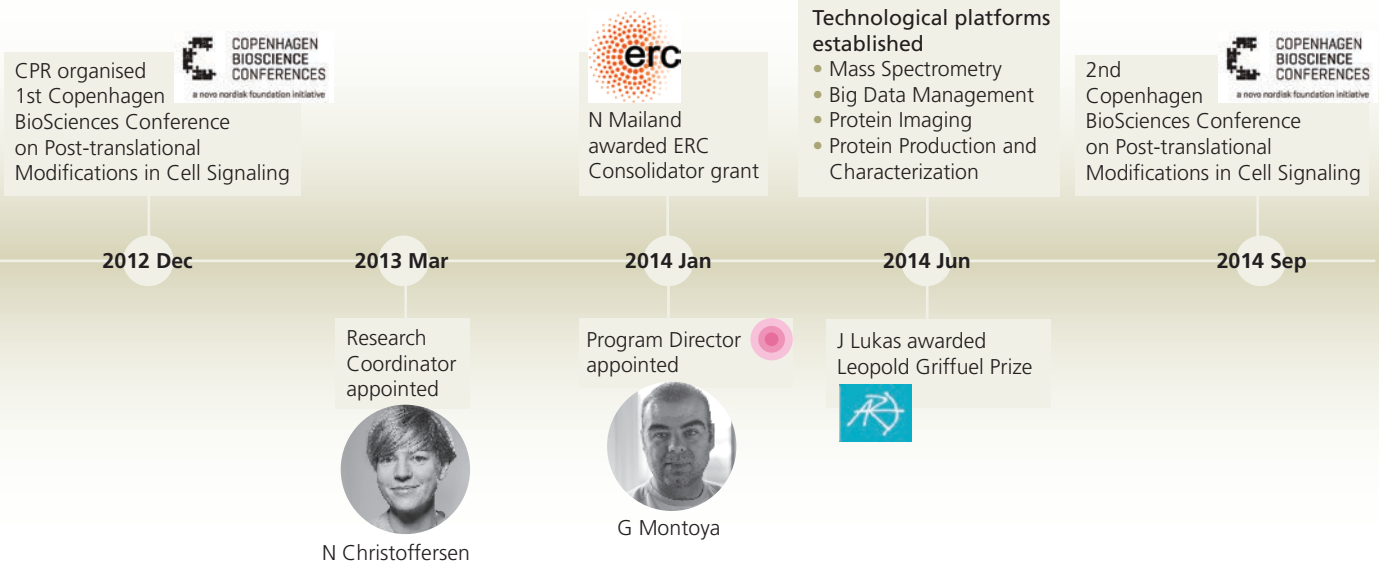
In line with our mission and vision, and to address the major open questions in protein science listed above, CPR has several features that, combined, make it unique on the world stage.

World-leading scientists

CPR is proud to have gathered a team of world-leading scientists to direct its four Programs in Proteomics (Matthias Mann), Protein Structure and Function (Guillermo Montoya), Protein Signaling (Jiri Lukas), and Disease Systems Biology (Søren Brunak), together with extremely talented young group leaders who have expertise spanning a broad spectrum of protein science and technology (see Figure 3).

CPR embedment in the Faculty

CPR is proud to be embedded in the Faculty of Health and Medical Sciences at UCPH. Here, CPR endeavors to promote 'win-win' interactions with the Faculty by integrating its activities with other centers of excellence at the Faculty and strengthening the Faculty's standing internationally. CPR also has a central role in the joint international PhD recruitment program and, for instance, helped initiate the 'thesis assessment committees' with our colleagues from DanStem, the NNF Center



for Basic Metabolic Research, and the NNF laureates.

Highly integrated and collaborative

CPR was conceived as a highly integrated research center, both internally and externally (see Figure 4 for examples of stakeholders). Internally, CPR scientists benefit from access to a unique combination of four integrated state-of-the-art protein technology platforms. CPR also promotes interactions locally within the NNF Center Cluster and with the Faculty. Externally, CPR is a hub that links and integrates first-rate, cutting-edge, and innovative technological, clinical, and scientific expertise from a wide range of national and international stakeholders. The global reach of CPR is also evident in the large number of collaborations we have established worldwide, and our participation in and organization of international conferences and symposia.

Protein-based 'precision medicine'

At CPR, we handle the entire value chain of protein research, connecting basic mechanistic protein biology and translational research to therapy and diagnostics. To this end, we have integrated into CPR the concept of protein-based 'precision medicine' based on sophisticated techniques in genomics and proteomics. This approach will lead to more specific understanding, diagnosis, treatment, and prevention of disease for individual patients. CPR is in a unique position to advance protein-based precision medicine because we develop individualized technologies to precisely study the mechanistic basis of disease and reveal new potential drug targets.

Training 'complete protein scientists'

A key element of the integrated nature of CPR, and one that distinguishes the

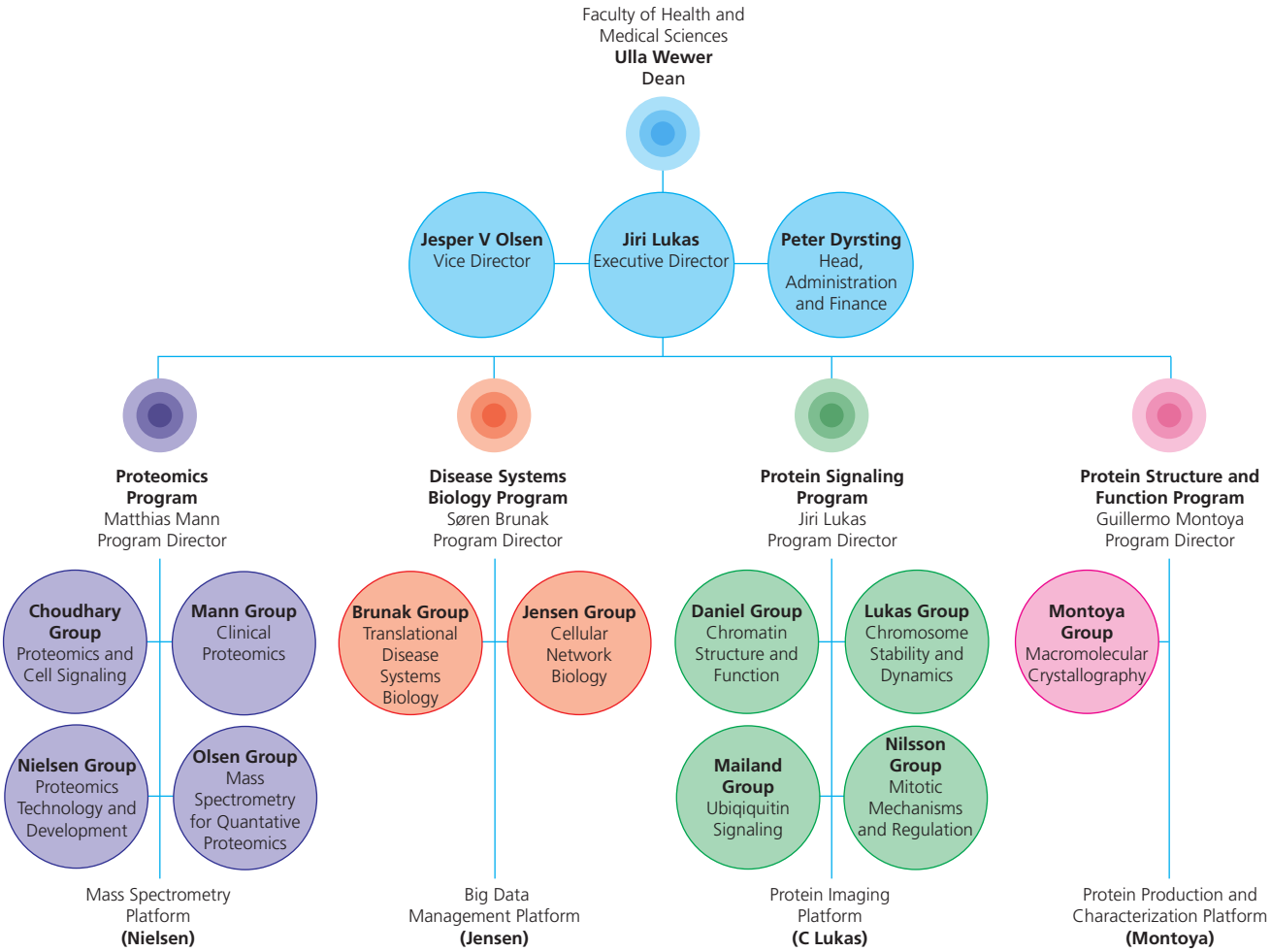


Figure 3 | CPR consists of four highly integrated scientific programs and technology platforms.

technological platforms of CPR from conventional core facilities, is that they are led by expert CPR scientists. This scientist-driven management of the technological platforms enables top-notch technological support and encourages CPR's students and postdocs to leave their analytical 'comfort zones' by exposing them to a wide range of state-of-the-art technologies.

This integrated approach also enables CPR to readily identify new cross-disciplinary projects and to foster the next generation of 'complete protein scientists.' Crucially, an easily accessible platform set-up also permits holistic protein investigations to be undertaken, and generally fosters close collaborations between research groups and maximizes synergy between programs. We feel that the success of this approach makes it attractive to be rolled-out on a larger scale in the Medicon Valley area.

As a result of this integrated approach, most research projects at CPR are already conducted as cross-program collaborations, in which protein-based mechanisms are elucidated using a combination of protein biophysics, microscopy, mass spectrometry, and bioinformatics. Moreover, interaction between the recently established Clinical Proteomics group with the Translational Disease Systems Biology group will enable CPR researchers to cover much of the value chain of protein research, from basic research to therapy and diagnostics.

OVERVIEW OF ACHIEVEMENTS IN 2015

While the detail of CPR's achievements in 2015 are described throughout this Annual Report, and selected highlights are illustrated in Figure 1, three main advances stand out.

The first is the reinforcement of the position of CPR's Programs and Platforms at the front-line of protein research by Søren Brunak's relocation of his big data research to CPR and the establishment of Matthias Mann's new clinical proteomics group, alongside the outstanding high-caliber team of senior and junior scientists. Together with the NNF's extension of CPR's core funding and extra external funding secured this year, we are perfectly placed to forge ahead with addressing the big questions in protein research.

The second is the impact made by CPR's scientific discoveries and innovations in 2015, as demonstrated by the 80 papers published, mostly in very high-impact journals.

The third is our continued effort to educate young scientists to become complete protein scientists. In 2015, five students completed their PhDs at CPR and moved on to the next stage of their career.

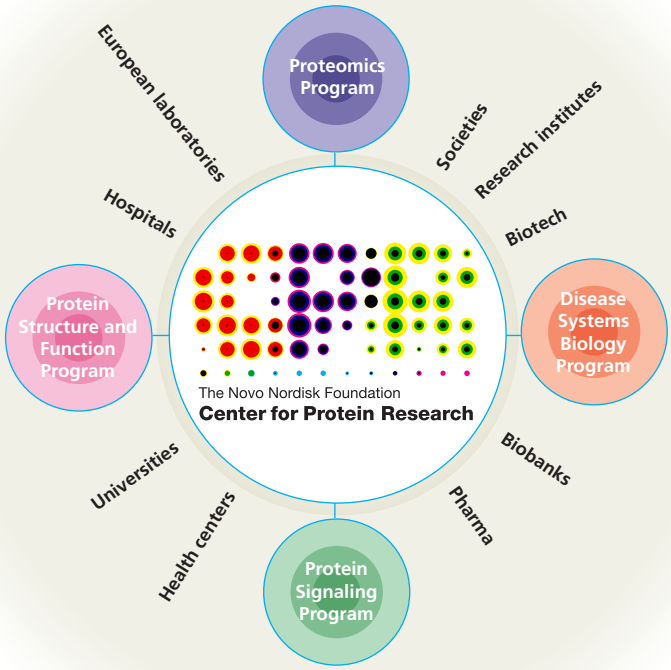


Figure 4 | CPR is a highly integrated research center, with both internal and external stakeholders.

GOVERNANCE AND ORGANIZATION

In 2015, the four research Programs and their corresponding technology Platforms, which were set up in the earlier years of CPR's existence, became successfully established, consolidated, and integrated. Some enhancing organizational developments in the Programs are worth highlighting here.

Of note this year, Matthias Mann started his own Clinical Proteomics group up in the Proteomics Program, of which he is Director, having secured funding from the Novo Nordisk Foundation (NNF). Together with the other three groups and the associated Mass Spectrometry Platform, the planned scale of the Proteomics Program has now been met. By the end of 2015 the program employed 29 staff members, and is expected to approach 40 team members by 2017 due to the addition of Matthias Mann's new group and a growing number of externally funded research projects.

Another major change in 2015, which is of great benefit to us, is that Søren Brunak relocated to work full time at CPR after more than 25 years at the Technical University of Denmark (DTU). With Søren Brunak fully on board, the development of the Disease Systems Biology Program and the corresponding Big Data Management Platform is complete, with an option to recruit a Junior Group Leader for the Program in 2016. By the end of 2015, 18 staff members were employed in Disease System Program, and this is expected to approach 30 by the end of 2016 due to an increasing number of externally funded research projects, primarily from the EU.

During 2015, the Protein Structure and Function Program, led by Guillermo Montoya, consisted of the Montoya group, the Wikström group, and the Protein Production and Characterization Platform. The platform is the largest at CPR and consists of three individual teams. Each team provides a different set of services and has its own team coordinator, the last of whom has recently been appointed. At the end of 2015, Group Leader Mats Wikström moved on to pursue a career in a US pharmaceutical company.

By the end of 2015, the Program hosted 22 staff members and its size is expected to remain the same over the next few years.

The fourth Program, Protein Signaling, led by Jiri Lukas, currently has four individual research groups, making the Program fully functioning. In 2015 the Flow Cytometry Facility, was established within the Protein Imaging Platform in collaboration with DanStem. Altogether, the Protein Signaling Program is the largest at CPR with 42 members onboard by 31 December 2015, and it is expected to remain at this level over the next year.

INTEGRATION INTO THE FACULTY OF HEALTH AND MEDICAL SCIENCES

CPR is an integrated part of the Faculty of Health and Medical Sciences at the University of Copenhagen. We have established numerous collaborations with groups at other departments at the Faculty. The embedment in the University is win-win, as our state-of-the-art technology platforms, in particular, make us attractive collaboration partners for researchers from other parts of the Faculty and, in turn, CPR researchers gain crucial biological and medical knowledge as well as access to state-of-the-art animal facilities and patient samples.

To further strengthen our involvement and commitment to the Faculty, we have recently appointed the first CPR Associate Member, Alicia Lundby, who left the Proteomics Program where she was a postdoc for a Group Leader position at the Department of Biomedical Sciences. This new type of position, which comes with privileged access to our technology platforms, is currently earmarked for former CPR postdocs who have become Group Leaders at Danish Universities or other NNF centers. Importantly, with the establishment of Matthias Mann's Clinical Proteomics group and Søren Brunak's move to a full-time role in his Translational Disease Systems Biology group in 2015, CPR has increased its translational work, for which our embedment in the Faculty is essential.

Another means by which we contribute to the Faculty is by organizing Masters and PhD courses in protein technologies, which include lectures provided by CPR experts and hands-on practical experience in our laboratories. Equally important is the monthly CPR seminar series, to which world-leading experts in areas of protein research are invited to CPR to present their latest research and interact with our students, postdocs, and Group Leaders. The seminars are open to all researchers from the Faculty and are advertised to other interested parties; they are usually completely packed.

"We are proud to be embedded in the Faculty of Health and Medical Sciences as the Faculty's strong focus on top research and education enables us to attract the best international talent and develop the next generation of protein scientists."

JESPER V OLSEN,
VICE DIRECTOR

STAFF AND RECRUITMENT

At the end of 2015, CPR employs 124 staff members from 29 different countries, compared with 119 staff at the end of 2014. Of these 124 employees, 20 started at CPR in 2015.

As in 2014, the gender balance across CPR is 50/50. At the management level we have two female leaders, Professor Claudia Lukas (Protein Imaging Platform) and Associate Professor Alicia Lundby (CPR Associate Member and Group Leader, Proteomics) among the 16 managers at CPR.

The number of scientific staff has been maintained in 2015 whereas the number of research support and administrative support members has increased by five. This increase in support staff was needed due to the launch of CPR's technological platform concept and a number of new large research projects.

Regarding the composition of the scientific staff by nationality, the number of staff coming from outside Europe has increased, supporting CPR's internationalization policy. See Figure 5 for an overview of the staff composition at CPR during 2015.

CENTER MANAGEMENT

CPR's executive management team consists of Ulla Wewer (Dean of the Faculty of Health and Medical Sciences) and the following members of CPR: Jiri Lukas (Executive Director, Program Director), Jesper V Olsen (Vice Director), Matthias Mann (Program Director), Søren Brunak (Program Director), Guillermo Montoya (Program Director), and Peter Dyrsting (Head of Administration and Finance).

The management team frequently interacts on three levels: (1) the Dean, Executive Director, and Vice Director typically meet twice a month to discuss strategic matters; (2) the Program Directors meet regularly to discuss and define scientific strategy, finances, and development for CPR; and (3) the Executive Director, Vice Director, and Head of Administration and Finance meet every week to streamline the day-to-day management of CPR.

In addition, Group Leader meetings take place six to eight times a year to ensure sharing of information and knowledge and to create a forum for discussion of CPR issues. At the Group Leader meetings, space is provided to give Student and Postdoc Association (SPA) representatives the opportunity to come up with suggestions and give their opinion on important strategic decisions.

"We are proud that we have achieved and maintained a 50/50 gender balance across CPR and we are now striving for a similar balance at management level. To do this, we are implementing measures that will help us attract a more balanced field of applicants when we search for highly qualified Group Leaders."

PETER DYRSTING,
HEAD OF ADMINISTRATION
AND FINANCE

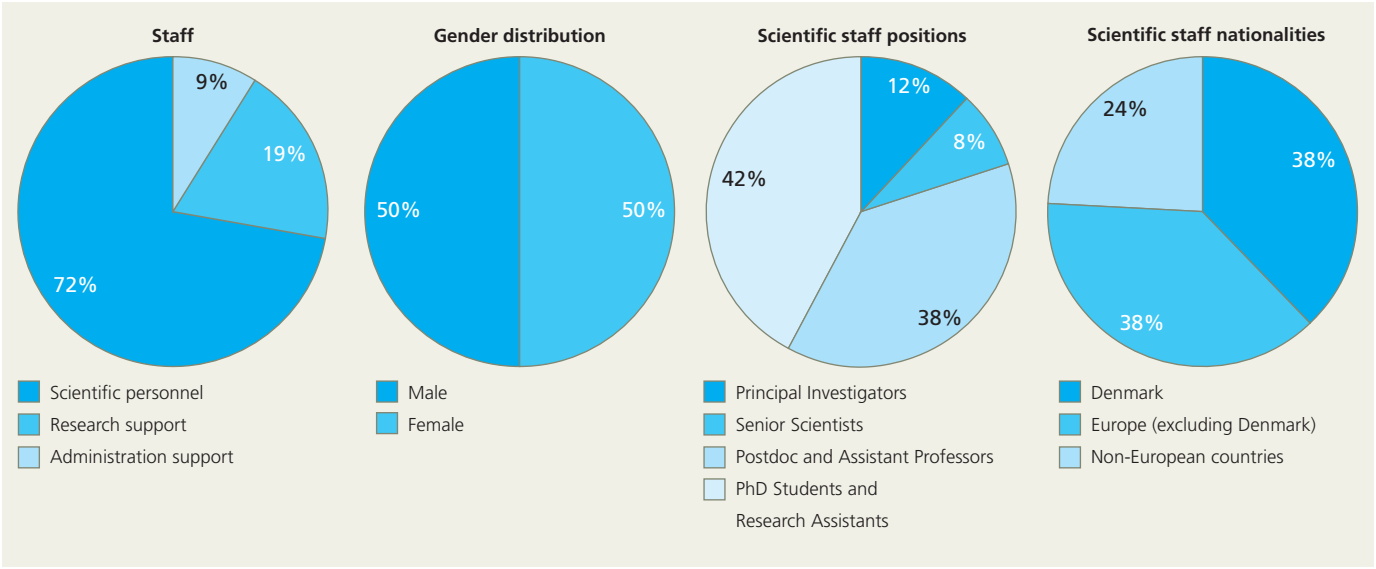


Figure 5 | Staff composition in 2015.

SCIENTIFIC ADVISORY BOARD

At CPR, we are fortunate to have a Scientific Advisory Board (SAB) consisting of highly prominent scientists, all of them renowned scientific authorities in their research fields (see below for a list of the 2015 SAB members and their background and expertise).

Every year our performance, productivity, innovations, and integration of the research Programs and technological Platforms are evaluated by the SAB, which gives advice on the strategic direction of CPR and the progress and development of the scientific program.

The CPR executive management team invites new board members to join the SAB and we are delighted that Professor Dame Janet Thornton became a member of our SAB in 2015. She is a physicist by training, and her research has focused on understanding protein structure, function, and evolution using computational approaches.

"We have been extremely impressed with all the positive changes that have been implemented at CPR this year and look forward to continued success in 2016."

Dr Tony Hyman (SAB Chair), Director at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden (Germany).

STUDENT AND POSTDOC ASSOCIATION

The Student and Postdoc Association (SPA) at CPR celebrated its first anniversary in 2015. In its first year, SPA has become a strong voice for students and postdocs at CPR and strives to strengthen the social and scientific networks among junior researchers at the center. SPA put forward a number of proposals during its first year to improve research seminars and organizational meetings at CPR, and all of these have been implemented with great success. Representatives of SPA regularly participate in the Group Leader meetings, and its own regular

2015 SAB members

Professor Torben Falck Ørntoft, Head, Department of Molecular Medicine, Aarhus University Hospital at Skejby (Denmark). *Translational cancer research.*

Professor Angus Lamond, Director, the Wellcome Trust Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee (UK). *Proteomics, advanced imaging.*

Professor Poul Nissen, Professor of Protein Biochemistry, Department of Molecular Biology and Genetics, Aarhus University (Denmark). *Structural biology.*

Dr Andre Nussenzweig, Chief at the Laboratory of Genome Integrity National Institutes of Health, Center for Cancer Research, National Cancer Institute, Bethesda (USA). *DNA damage response, mouse models of genome instability disorders.*

Professor Ivan Dikic, Director of the Institute of Biochemistry, Goethe University, Frankfurt and Scientific Director of the Frankfurt Institute for Molecular Life Sciences (Germany). *Ubiquitin signaling.*

Professor Dame Jane Thornton, Director Emeritus of the European Molecular Biology Laboratory European Bioinformatics Institute (EBI) and Senior Scientist, EBI, Cambridge (UK). *Protein structure, function, and evolution using computational approaches.*

"The SPA is clearly an asset to CPR."

TOM MISTELI

"CPR is a remarkable center, doing outstanding science, with a highly collaborative and interactive style, which is hard to match."

DAVID ALLIS

lunch meetings and Friday get-togethers are a great opportunity to get to know colleagues from other programs and groups, discuss future plans, and hear about the career paths of CPR's Group Leaders.

In 2015, with full support from CPR's management, SPA greatly expanded its activities. In the spring, SPA organized a scientific writing workshop with Elevate Scientific, which received such positive feedback from PhD students and postdocs alike that this will now become a regular event at CPR. SPA also had the great pleasure of inviting and welcoming two outstanding scientists to give talks at CPR, Tom Misteli (National Cancer Institute, USA) and David Allis (Rockefeller University, USA). Both speakers thoroughly enjoyed their visits, as did all of SPA and the other attendees of the fascinating and inspiring seminars. In the summer of 2015, SPA hosted it's first SPA retreat, which had a good balance of scientific and social activities, and we received enthusiastic feedback. More than 20 researchers presented their work, illustrating the diversity of CPR's research and leading to fruitful discussions that have helped to identify new ways for us to work together and make the best use of our time at CPR. In late 2015, four newly elected SPA members joined the three continuing SPA representatives, and SPA continues to shape CPR and ensure a positive and dynamic experience for all students and postdocs who work here.

SPA representatives

CENTER ADMINISTRATION

CPR's administration team provides administrative and service support to the entire center. The team consists of 12 members and provides close support to all research groups, as well as the management (11 of them pictured, Kateryna Kolkova is Nanna Christoffersen's maternity cover).

The support for the research groups covers a wide range of tasks, including purchasing, day-to-day support for group members and the running of the laboratories, and meeting organization. In addition, Group Leaders and grant recipients receive support for publications and other communications, project coordination, health and safety, recruitment, and finances.

Administration team

The support for the senior management consists of strategic planning and counseling, research coordination, communication, recruitment, human resources, finances, and project coordination.The administration group is, in turn, supported at both Faculty and University levels mainly in terms of legal assistance, human resources, communication, computing, and accounting.

PROTEOMICS PROGRAM

Proteomics and Cell Signaling
CHOUDHARY GROUP

Clinical Proteomics
MANN GROUP

Proteomics Technology Development
NIELSEN GROUP

Mass Spectrometry for Quantitative Proteomics
OLSEN GROUP

Mass Spectrometry Platform

FROM THE PROGRAM DIRECTOR
MATTHIAS MANN

"We continually develop cutting-edge proteomic technologies to analyze cellular processes relevant to health and disease."

The Proteomics Program is a world leader in the development of proteomics technologies and their innovative use in cell biology and disease biology. As I am also based at the Max Planck Institute of Biochemistry in Munich, Germany, we have an effective synergy with this institute and a large critical mass in most areas of proteomics.

Organizational developments

The Program now consists of four groups that are led by Jesper V Olsen, who is also Vice Director of CPR, Chunaram Choudhary, Michael L Nielsen, who is also Leader of the Proteomics Platform, and myself. The Program prides itself on continually pushing the technologies to make more and more areas amenable to proteomics analysis. While we cover a wide range of proteomics technologies, we particularly focus on post-translational modifications (PTMs), which are central to cellular decision-making in health and disease.

Technological advances

In 2015, many of our technology developments were translated into novel insights into important biological questions. This tight and successful integration of large-scale data generation with the elucidation of actual biological mechanisms sets us on the right path for future discoveries and insights. For example, we developed and implemented a powerful technology for peptide pre-separation and novel mass spectrometry instrumentation.

Another major development in 2015 was the establishment of the Clinical Proteomics Group with a 60 million DKK (8 million EUR) grant from the Novo Nordisk Foundation (NNF). With this generous grant, we will develop technologies that enable the rapid analysis of the blood proteome; a potential game changer in medical diagnostics and, indeed, in the 'phenotyping' of patients and healthy individuals.

Research highlights

Notable highlights in 2015 include the first 'proteomic movie' of the important process of repair of DNA interstrand crosslinks, which was the result of a close collaboration between the Mann group, the Max Planck Institute, and Niels Mailand's group at CPR, and was published in *Science* (Räschle *et al.*, *Science*, 2015). Breakthrough insights by the Choudhary group into the network of deacetylases, which have pivotal functions in metabolism and other processes, led to a publication in *Nature Biotechnology* (Schölz *et al.*, *Nat Biotechnol*, 2015). The Olsen group unraveled a mechanism of cellular decision-making in neuroblastoma (published in *Science Signaling* — Emdal *et al.*, *Sci Signal*, 2015 — and highlighted on the cover of the journal). In addition, the Nielsen group performed a systems-wide investigation of the cellular effects of biotin starvation as part of its work on less studied protein modifications (Madsen *et al.*, *Nat Commun*, 2015).

International standing and collaborations

Internally, the group has established strong collaborations with virtually every research group in the field, helping to give these groups a crucial competitive edge in research and in attracting grants. Cooperation in the Copenhagen area includes other NNF centers, Eske Willerslev's group at the Center for GeoGenetics, University of Copenhagen (UCPH), as well as companies such as Novo Nordisk A/S and other organizations located in the Medicon Valley area. Internationally, besides the strong connection with the Max Planck Institute, we work closely with signaling groups at the University of Cambridge and many other prestigious universities worldwide.

"We combine high-resolution mass spectrometry and genome engineering to systematically investigate acetylation and ubiquitylation signaling networks."

PROTEOMICS AND CELL SIGNALING

CHOUDHARY GROUP

Professor
Chunaram Choudhary (Group Leader)

Associate Professor
Brian T Weinert

Postdocs
Thomas Wild
Rajat Gupta
Takeo Narita
Magdalena Budzowska
Christian Schöltz
Vytautas Lesmantavicius

PhD Student
Shankha Satpathy

Technician
Elina Maskey

INTERNAL COLLABORATORS

Niels Mailand (Mailand group, Protein Signaling Program) proteomic analysis of proteins involved in the maintenance of genome integrity.

Lars J Jensen (Jensen group, Disease Systems Biology Program) bioinformatics analysis of proteomic datasets.

Jakob Nilsson (Nilsson group, Protein Signaling Program) understanding the mechanisms of APC/C activation.

Jeremy A Daniel (Daniel group, Protein Signaling Program) proteomic analysis of proteins involved in class-switch recombination.

Claudia Lukas and **Jiri Lukas** (Lukas group, Protein Signaling Program) identification of novel factors involved in DNA damage signaling.

EXTERNAL COLLABORATORS

Ian D Hickson [University of Copenhagen (UCPH), Denmark] proteomic analysis of proteins involved in DNA replication.

Matthias Mann and **Juergen Cox** (Max Planck Institute of Biochemistry, Germany) development of proteomic methods and computational analysis of proteomic datasets.

Rudi Zechner (University of Graz, Austria) metabolic regulation of lysine acetylation.

Frank Buchholz and **Francis Stewart** (University of Dresden, Germany) proteomic analysis of stem cells.

Michael Lisby (UCPH, Denmark) identifying novel factors in DNA damage signaling.

James E Bradner and **Patrick Matthias** (Harvard Medical School, USA, and Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland) identification of cellular targets of lysine deacetylase inhibitors.

Jonathan Weissmann (University of California, San Francisco, USA) unraveling the mechanisms of proteasome inhibitor resistance.

Steve Cohen (UCPH, Denmark) investigating the function of ubiquitylation in HIPPO signaling.

2015

Challenges and research aims

Lysine acetylation and ubiquitylation are recognized as important regulatory protein PTMs in eukaryotes. While their biological importance is clearly established, our knowledge about the dynamics of these modifications and the enzymes involved in their regulation is far from complete. Our group develops mass spectrometry-based technologies to tackle these challenges. To that end, we have implemented the latest genome editing technologies to generate cell models, and plan to combine this with our quantitative proteomic approach to study acetylation and ubiquitylation signaling at a systems level. Furthermore, we work closely with our in-house and external collaborators to systematically mine datasets and to identify novel regulatory functions of these modifications.

STRATEGIC GOALS

- To develop novel proteomic approaches to estimate the stoichiometry of lysine acetylation sites
- To investigate the cellular targets of lysine deacetylase inhibitors
- To investigate signaling networks of the B cell receptor

Achievements

A major achievement this year was the systematic elucidation of the in vivo specificities of lysine deacetylase inhibitors. Although, lysine deacetylase inhibitors are widely used in basic research and in treating patients, which acetylation sites are affected by specific inhibitors has largely remained unknown. This work, by postdoc Christian Schölz, has provided for the first time a global view of acetylation sites affected by widely used deacetylase inhibitors in human cells (see Figure 6). This study has also revealed the possible mechanistic basis of how bufexamac caused pro-inflammatory reactions in patients. Notably, marketing approval for this anti-inflammatory drug was withdrawn in 2010 due to its pro-inflammatory side effects. This work was published in *Nature Biotechnology* (Schölz *et al.*, *Nat Biotechnol*, 2015).

In another major project, we developed a novel method to estimate the stoichiometry of acetylation on individual sites and investigated how acetylation is affected by metabolism. Based on this work, we propose that acetylation of mitochondrial proteins is mostly caused by a non-enzymatic mechanism. Such acetylation may function as a 'protein lesion,' and sirtuin type deacetylases in mitochondria may play a 'housekeeping' role by rescuing proteins from acetyl lesions. This work, spearheaded by Brian Weinert, helps our understanding of how metabolism affects acetylation and its implications in regulating protein function. The work was published in *EMBO Journal* (Weinert *et al.*, *EMBO J*, 2015) and was highlighted in its 'News & Views' section.

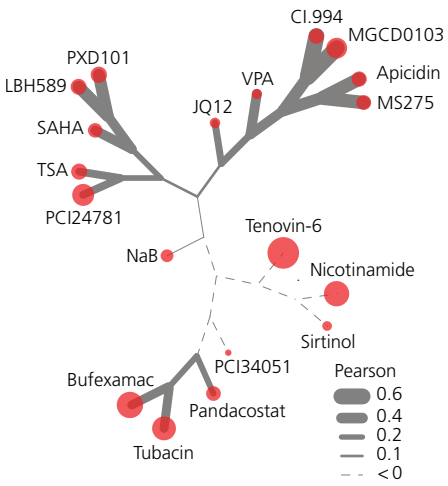


Figure 6 | Clustering of lysine deacetylase inhibitors based on their in vivo acetylation patterns in human cells. Node size reflects number of upregulated sites and line thickness corresponds to the degree of correlation (Schölz *et al.*, *Nat Biotechnol*, 2015).

Another highlight of 2015 was a project that used a multi-layered proteomic approach to monitor the dynamics of B cell receptor (BCR) signaling complexes (signalosomes that initiate and transmit the signal to downstream effectors) and the dynamics of downstream phosphorylation and ubiquitylation signaling. BCR is essential for the development and function of B cells. We showed that BCR-induced phosphorylation of RAB7A at amino acid S72 prevents its association with effector proteins and with endolysosomal compartments. In addition, we showed that BCL10 is modified by linear ubiquitin assembly complex (LUBAC)-mediated linear ubiquitylation, and demonstrated an important function of LUBAC in BCR-induced NF-κB signaling. This work, by PhD student Shankha Satpathy and a former postdoc in the lab, Sebastian Wagner, was published in *Molecular Systems Biology* (Satpathy *et al.*, *Mol Syst Biol*, 2015) and was highlighted in *Science Signaling*. These results offer a global and integrated view of medically important BCR signaling and provide valuable dataset resources to the community.

"We aim to revolutionize medical diagnostics through rapidly quantifying the plasma proteome – the collection of proteins circulating in the blood."

CLINICAL PROTEOMICS

MANN GROUP

Professor
Matthias Mann (Group Leader)

PhD Student
Phillip Geyer (shared with Max Planck Institute of Biochemistry, Munich, Germany)
Nicolai Jacob Wewer Albrechtsen (shared with Department of Biomedical Sciences, UCPH, Denmark)

Postdoc
Atul Shahaji Deshmukh

INTERNAL COLLABORATORS

Christian Schölz, **Brian Weinert** and others (Choudhary group, Proteomics Program) acetylation.

Jesper V Olsen and **Michael Lund Nielsen** (Proteomics Program) technology development for proteomics.

Sune Pletscher-Frankild (Jensen group, Disease Systems Biology Program) the proteomic and bioinformatics analysis of the class I peptidomes.

Niels Mailand's group, (Protein Signaling Program) the proteomic and cellular analysis of DNA damage repair pathways.

Diego Iglesias-Gato (Morales group, formerly Protein Signaling Program, now Danish Cancer Society) prostate cancer proteomics.

EXTERNAL COLLABORATORS

Department of Proteomics and Signal Transduction, the Max Planck Institute of Biochemistry (Munich, Germany) a wide range of technological and cell biological questions.

Camilla Scheel and **Bente Klarlund Pedersen** (Rigshospitalet, Copenhagen, Denmark), as well as researchers from Novo Nordisk A/S, secreted factors from brown fat.

Martin Ridderstrale and **John Nolan** (Steno Diabetes Center, Gentofte, Denmark) the health status of diabetic patients as revealed by plasma proteomics.

2015

Challenges and research aims

Our newly established Clinical Proteomics group will undertake an ambitious new biomedical research program on proteomics of blood/plasma for patient phenotyping. We aim to identify biological markers for diabetes, obesity, and other metabolic disorders that can be used to improve diagnosis and develop personalized therapies. Our overarching goal is to prevent the development of the metabolic syndrome by targeted and personalized lifestyle interventions.

The Clinical Proteomics group works in close collaboration with Matthias Mann's group at the Max Planck Institute of Biochemistry, Munich, Germany. Furthermore, the group will be tightly integrated with Søren Brunak's Translational Disease Systems Biology group in areas such as 'big data' analysis and mining electronic health records.

STRATEGIC GOALS

- To develop and deploy a plasma proteomics pipeline capable of identifying and quantifying about 1000 different proteins in human plasma
- To complete the first small and medium scale plasma proteomics studies
- To develop expertise and recruit staff to implement the plasma proteomics pipeline

A primary goal is to discover novel blood protein biomarkers that can be used for the diagnosis and possibly the prevention of type 2 diabetes, obesity, and other metabolic diseases (see Figure 7). We will develop a platform to analyze the proteomics of up to 100 individuals per day at great accuracy and depth of coverage. Together with our partners at the Steno Diabetes Center, Rigshospitalet, the Faculty of Health and Medical Sciences at UCPH, the NNF Center for Basic Metabolic Research, and elsewhere, we will apply this platform to characterize the blood proteome and how it is associated with known and novel metabolic risk factors for diabetes and cardiovascular disease.

Achievements

During the last year, we have undertaken a great deal of technology development, which is necessary to bring our vision of plasma proteomics to fruition. Key developments include a robust sample preparation technology that allows us to quantify the entire panel of apolipoproteins (important cardiovascular/metabolic risk markers). We also completed the first medium scale plasma proteomics study. Finally, we were awarded 60 million DKK (8 million EUR) for our activities as a result of our application for a large research program grant from the NNF in 2015.

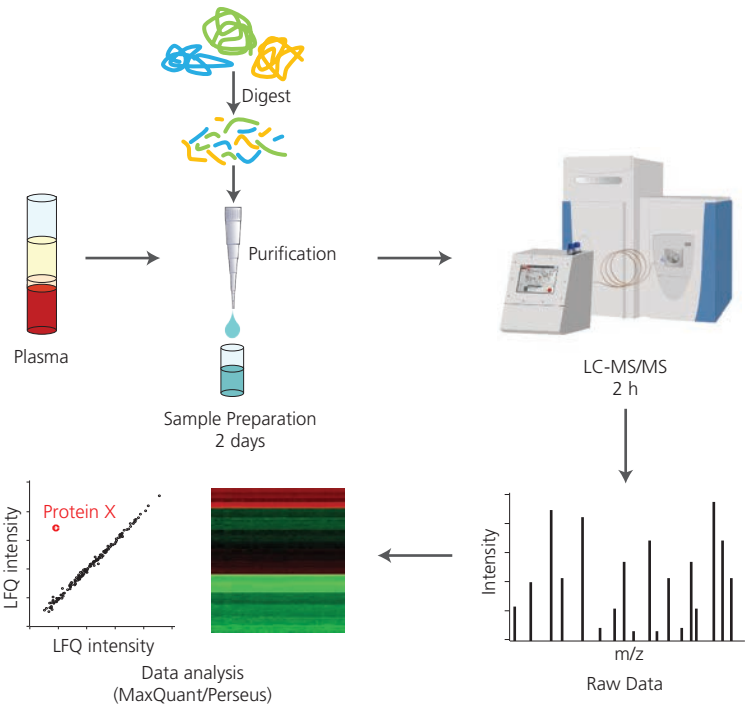


Figure 7 | Representation of the clinical proteomics workflow for rapid plasma analysis. A single drop of blood from a finger prick is processed and the resulting peptides derived from proteins in the blood are analyzed by mass spectrometry to help identify biomarkers.

"By exploiting our cutting-edge mass spectrometry facilities and experience, we aim to develop novel proteomics methods for a better understanding of currently uncharacterized post-translational modifications."

PROTEOMICS TECHNOLOGY AND DEVELOPMENT

NIELSEN GROUP

Professor
Michael L Nielsen (Group Leader)

Postdocs
Clifford Young
Kathrine B Sylvestersen
Ivo Hendriks
Niels Henning Skotte

PhD Students
Sara C Larsen
Meeli Mullari
Rosa Jersie-Christensen

Master's Student
Maria V Madsen

INTERNAL COLLABORATORS

Jiri Lukas (Lukas group, Protein Signaling Program) the role of protein modification by poly(ADP)-ribosylation in the genotoxic stress response.

Jesper V Olsen (Olsen group, Proteomics Program) the role of protein arginine methylation in the EGF signaling pathway and general proteomics technology development.

Jeremy Daniel (Daniel group, Protein Signaling Program) establishing novel proteomics technologies to characterize H2AX-interacting proteins in the DNA damage response.

Jakob Nilsson (Nilsson group, Protein Signaling Program) proteomic analysis of the mitotic checkpoint complex.

Niels Mailand (Mailand group, Protein Signaling Program) characterization of protein interactors in the DNA damage response.

Lars Juhl Jensen (Jensen group, Disease Systems Biology Program) bioinformatic analysis of poly(ADP)-ribosylation and arginine methylation.

Claudia Lukas (Protein Imaging Platform) providing imaging support.

EXTERNAL COLLABORATORS

Andre Nussenzweig (National Cancer Institute/ National Institutes of Health, USA) poly(ADP)-ribosylation in cancer.

Tony Kouzarides (Gurdon Institute, Cambridge, UK) histone arginine methylation and arginine citrullination.

Michael O Hottiger (Institute of Veterinary Biochemistry and Molecular Biology, Switzerland) characterization of poly(ADP)-ribosylation in human cells.

Manuel Stucki (Institute of Veterinary Biochemistry and Molecular Biology, Switzerland) proteomic analysis in the DNA damage response.

Philip M Iannaccone (Northwestern University, USA) characterization of GLI1-interacting proteins.

Michael Lisby [University of Copenhagen (UCPH), Denmark] lysine biotinylation in yeast.

Gonçalo Castelo-Branco (Karolinska Institute, Sweden) citrullination in human cells by the enzyme peptidylarginine deaminase (PADI) 2.

Maria Christophorou (Institute of Genetics and Molecular Medicine, Edinburgh, UK) citrullination in human cells by the enzyme PADI4.

2015

Challenges and research aims

Proteins engage in a wide array of molecular events in which the ability to discriminate between functional and non-functional interactions is crucial. Although the cellular 'modificome' is highly complex, only a small subset of the estimated 200 possible types of PTMs are currently amenable for analysis on a global scale. These include phosphorylation, ubiquitylation, and acetylation. Developing technologies for the comprehensive analysis of 'not-so-well-characterized' PTMs is a priority in our group, as such methods are required to better understand the complex nature of mammalian signaling events.

Our group is ideally positioned to address these challenges because of our extensive knowledge and experience in developing state-of-the-art proteomics technologies. To strengthen our analytical and biological capabilities for analyzing PTMs, we have recruited two postdocs (Ivo A Hendriks and Niels Henning Skotte) with proven track records in PTM analysis. We have also furthered our long-standing collaboration with Professor Tony Kouzarides in the UK, allowing us to pool our knowledge and resources to characterize the human arginine citrullinome, with the long-term aim of understanding the functional consequences of this PTM.

The physiological roles of citrullination include transcriptional regulation, the modulation of chromatin condensation associated with the innate immune response to infection, regulation of pluripotency, and skin homeostasis. Importantly, deregulation of PADI activity is a feature of diverse pathologies such as autoimmunity, cancer, neurodegenerative disorders, thrombosis, and prion diseases, while emerging evidence suggests that it is involved in the etiology of some of these disorders. However, unbiased methodologies for characterizing which proteins become targeted by PADI enzymes have been lacking until now. Utilization of the methodology developed in our group will enable proteome-wide characterization of citrullination and the better elucidation of its physiological roles, thereby yielding new mechanistic insights into its diverse cellular functions.

STRATEGIC GOALS

- To further develop our proprietary methods to study the role of arginine methylation in human cells and biological processes
- To increase our understanding of poly(ADP)-ribosylation in DNA damage responses using mass spectrometry
- To characterize arginine methylation and poly(ADP)-ribosylation in RNA and DNA metabolism, and elucidate how these modifications alter interactions with RNA and DNA in cells

Achievements

A key achievement this year was the comprehensive analysis of cellular defects caused by the essential vitamin and PTM biotin. Biotin is a covalent and tenaciously attached prosthetic group in several carboxylases that have important roles in the regulation of energy metabolism. Cellular biotin starvation is already known to cause inefficient energy expenditure, ultimately leading to a state of metabolic disorder, but the molecular mechanisms involved are not fully understood. Our analysis showed that biotin starvation causes a major energy switch by shuttling acetyl-CoA into the mitochondria, which results in mitochondrial protein hyperacetylation (see Figure 8).

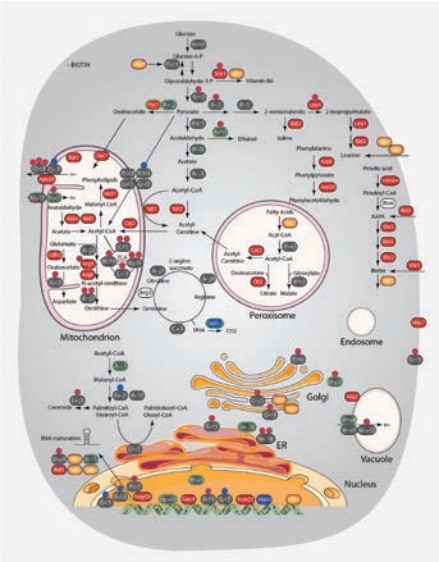


Figure 8 | The cellular consequences during biotin starvation.

Using high-resolution mass spectrometry, we demonstrated that this switch has widespread downstream effects on cellular respiration and redox balance. Oxidative stress is known to result in increased insulin sensitivity and contributes to the progression of various human diseases, including type 2 diabetes, in which an increased supply of energy substrates results in excessive mitochondrial reactive oxygen species (ROS). Our findings link biotin starvation and ROS production in eukaryotic cells, and delineate the effects of biotin availability on insulin sensitivity through acetyl-CoA flux and mitochondrial hyperacetylation. This research was published in *Nature Communications* (Madsen *et al.*, *Nat Commun*, 2015).

"With our world-leading expertise in phosphoproteomics and quantitative mass spectrometry, we aim to further understand cellular phospho-signaling networks following activation by extracellular stimuli or stress."

MASS SPECTROMETRY FOR QUANTITATIVE PROTEOMICS

OLSEN GROUP

Professors
Jesper V Olsen (Group Leader)
Alicia Lundby (CPR Associate Member and Associate Professor)

Postdocs
Chiara Francavilla
Christian Kelstrup
Louise von Stechow
Stephanie Munk

PhD Students
Alexander Hogrebe
Anna-Kathrine Pedersen
Christian Cramer (Industrial PhD with Novo Nordisk)
Jon Otti Sigurdsson
Kristina Bennet Emdal (PhD , now at Massachusetts Institute of Technology, Boston, USA)
Moreno Papetti
Rosa Jersie-Christensen
Tanveer Batth

Master's Students
Casper Konnerup

Research Assistants
Anna Eleonora Linscheid
Pi Camilla Poulsen

INTERNAL COLLABORATORS

Lars Juhl Jensen (Jensen group, Disease System Biology Program) analysis of kinase–substrate relationships.

Jiri Lukas (Lukas group, Protein Signaling Program) ATR and ATM signaling in replication stress and the DNA damage response.

Jakob Nilsson (Nilsson group, Protein Signaling Program) phosphatase substrates.

Guillermo Montoya (Montoya group, Protein Structure and Function Program) kinase assays and kinase–substrate relationships.

Niels Mailand and **Simon Bekker-Jensen** (Mailand group, Protein Signaling Program) SUMO signaling in DNA damage and MAPKAP kinase signaling during replication stress.

Michael Lund Nielsen (Nielsen group, Proteomics Program) development of proteomics technologies.

EXTERNAL COLLABORATORS

Josh Brickman [DanStem, University of Copenhagen (UCPH), Denmark] phosphoproteomics of embryonic stem cell differentiation.

Eske Willerslev and **Enrico Cappellini** (Center for GeoGenetics, UCPH, Denmark) paleoproteomics.

Alfred Vertegaal (Leiden University, the Netherlands) SUMOylation in the cell cycle and DNA damage response.

Blagoy Blagoev (University of Southern Denmark, Odense, Denmark) growth factor signaling.

Prasad Jallepalli (Memorial Sloan-Kettering, New York, USA) kinase–substrate target identification of cell cycle-regulated kinases.

Ugo Cavallaro [European Institute of Oncology (IEO), Italy] cancer signaling and tumor analysis.

Torben Ørntoft and **Claus Lindbjerg Andersen** (Aarhus University Hospital, Denmark) human tissue analysis.

Giulio Superti-Furga [Research Center for Molecular Medicine of the Austrian Academy of Sciences (CeMM), Vienna, Austria] phosphoproteomics of synergistic drugs for the treatment of childhood cancers.

Andres Lopez-Contreras (Center for Chromosome Stability, UCPH, Denmark) identification of BRCA targets.

2015

Challenges and research aims

The mechanisms and dynamics of phospho-signaling pathways in response to extracellular cues are still not fully understood, even though deregulation often results in diseases such as cancer and diabetes. Fundamental issues such as the functional selectivity of different receptor tyrosine kinase ligands activating the same receptor, which dictate the signaling duration, receptor endocytosis, and cellular outcome, are still to be addressed.

Our group is well placed to tackle these challenges with our in-house, state-of-the-art Proteomics Platform and in-depth knowledge of mass spectrometry techniques (Christian Kelstrup, Dorte Bekker-Jensen, and Rosa Jersie-Christensen), phosphoproteomics sample preparation methods (Tanveer Batth, Alicia Lundby, and Stephanie Munk), and cellular phospho-signaling biology (Chiara Francavilla, Kristina Emdal, and Louise von Stechow). In 2015, we recruited Alexander Hogrebe, who brings to the group his expertise in large-scale quantitative phosphoproteomics screens from the research group of Bernhard Küster in Munich, Germany. The focus of Hogrebe's PhD project will be PBK kinase signaling in breast cancer. We also have an established collaboration with Ugo Cavallaro (IEO, Italy) and Torben Ørntoft (Aarhus University, Denmark) that has allowed us to pool our knowledge and resources to study phospho-signaling networks in human tissue samples, with the long-term view of identifying cancer biomarkers and cellular signaling nodes that can be targeted by small molecule inhibitors.

We want to push the limits of these technologies for deep proteome profiling and global analyses of PTMs such as site-specific phosphorylation. We are developing quantitative interaction proteomics and PTM screens to analyze tissue samples from cell lines, rodent models, and patient materials such as biopsy samples.

STRATEGIC GOALS

- To develop and apply mass spectrometry-based proteomics technologies to study the workings of cellular phospho-signaling networks induced by extracellular stimuli or stress
- To characterize the role of the nerve growth factor-stimulated TrkA signaling pathway in neuronal differentiation and to elucidate how it is subverted in neuroblastoma

Achievements

In 2015, we published several papers with our local and international partners, including Eske Willerslev (UCPH) and a group of international evolutionary anthropologists, with whom we used our mass spectrometry-based proteomics technologies to sequence ancient proteins and thereby resolve the evolutionary mystery about the origin of Darwin's South American ungulates (Welker *et al*., *Nature*, 2015). We also continued our long-standing collaboration on SUMOylation with Alfred Vertegaal (the Netherlands) and published a proteomics screen of SUMO-2 regulation of chromatin modifiers in response to DNA damage. (Hendriks *et al*., *Cell Rep*, 2015).

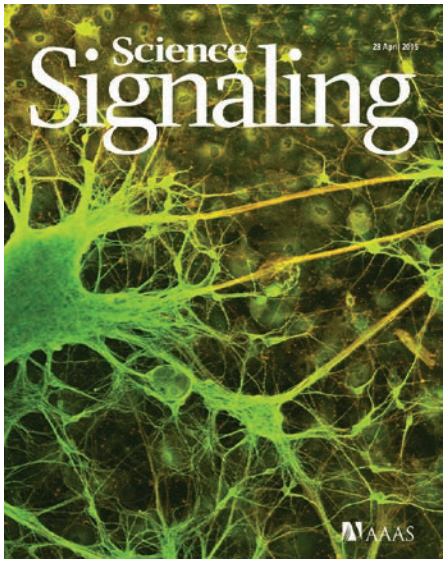


Figure 9 | The cover of *Science Signaling* showing cultured neurons featured our Research Article (Emdal *et al*., *Sci Signal*, 2015), in which we used temporal proteomics to identify a control mechanism of neuroblastoma cell differentiation.

Another key achievement this year was the discovery of an inhibitory role of an E3 ubiquitin ligase in controlling the level of the receptor tyrosine kinase TrkA in neuroblastoma cells. TrkA is the high-affinity receptor for nerve growth factor (NGF) and its expression level generally correlates with good prognosis for neuroblastoma patients. We used quantitative proteomics to analyze the temporal changes in the TrkA interactome, phosphoproteome, and proteome of TrkA-expressing neuroblastoma cells stimulated by NGF. We discovered that the E3 ligase Cbl-b controlled TrkA levels and that knockdown of Cbl-b strongly promoted neuronal differentiation. This work, by PhD student Kristina Emdal and postdoc Chaira Francavilla, is exciting because Cbl-b expression levels could serve as a biomarker for neuroblastoma classification and as a potential therapeutic target. The research was published in *Science Signaling* (Emdal *et al*., *Sci Signal*, 2015) and highlighted on the cover of the journal (see Figure 9).

MASS SPECTROMETRY PLATFORM

Professor
Michael L Nielsen, Platform Leader

Mass Spectrometry Specialist
Dorte Breinholdt Bekker-Jensen (maternity leave)
Rosa Jersie-Christensen (maternity leave cover)

Student Helper
Sofie Føns

The Mass Spectrometry Platform was established in 2014 and its aim is to ensure that CPR retains world-leading mass spectrometry technology, provide technical support and maintenance for the Proteomics Program, and provide analytical proteomics support for all CPR research groups and our collaborators.

During 2015, the Clinical Proteomics group and the Mass Spectrometry Platform invested in a Thermo Scientific Orbitrap Fusion Lumos Tribrid mass spectrometer (Figure 10), a top-of-the-line mass spectrometer that enables data gathering and analysis at unprecedented speed and sensitivity as it incorporates the brightest ion source available on any commercial instrument. Moreover, the instrument contains a segmented quadrupole mass filter with improved selectivity and ion transmission, advanced vacuum technology for improved ion transmission to the Orbitrap mass analyzer, and higher capacity electron transfer dissociation (ETD) fragmentation. Collectively, these improvements allow our researchers to excel in the most challenging analytical applications, including comprehensive analysis of low level PTMs, data independent acquisition, multiplexed relative quantitation using isobaric tags, and intact protein characterization, as well as multiple tandem-mass spectrometric (MSn) analyses. With the improved analytical capabilities that the Orbitrap Fusion Lumos provides, we have ensured that the Mass Spectrometry Platform is able to perform state-of-the-art proteomics research at the highest international level, both now and in the future, and that we are equipped to tackle the most interesting and complex biological applications.

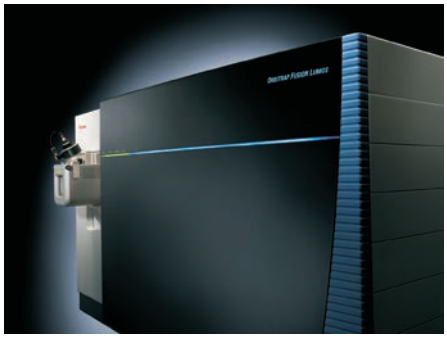


Figure 10 | The Orbitrap Fusion Lumos mass spectrometer.

DISEASE SYSTEMS BIOLOGY PROGRAM

Translational Disease Systems Biology
BRUNAK GROUP

Cellular Network Biology
JENSEN GROUP

Big Data Management Platform

FROM THE PROGRAM DIRECTOR
SØREN BRUNAK

"We focus on detailed phenotyping of individual patients, to take into account not only the full spectrum of disease comorbidities but also their detailed development over time."

Patients often suffer from more than one disease in various chronic conditions. It is therefore important to not focus on one disease in isolation, but to conduct deep phenotyping of individual patients to get a clear picture of all disease comorbidities over long periods of time. We do that in this program and, for patient subgroups, we are particularly interested in human proteome variation and how this variation impacts underlying disease etiologies and rewiring at the network biology level. To that end, we use and develop computational techniques for the joint analysis of molecular and clinical data, as well as data from the published literature.

Organizational development

The Program consists of two groups: Cellular Network Biology (Lars Juhl Jensen) and Translational Disease Systems Biology (Søren Brunak). We are also responsible for the Big Data Management Platform, which provides supercomputer resources and advanced storage schemes to research projects across CPR and its collaborative stakeholders.

The Program has a strong focus on the role of proteins, in terms of molecular level network biology operating at the cellular, organ, or organismal levels. This includes the localization of molecular entities, such as protein complexes, in tissues and subcellular compartments (Jensen group). The Brunak group focuses on the tissue specificities of disease, including the detailed understanding of comorbidities and adverse reactions to therapeutic agents, and integration of clinical data from individuals. This approach may reveal proteins that could be developed as drugs or drug targets, explain disease comorbidities, or rationalize adverse drug reactions by taking human proteome variation into account.

Research highlights

In 2015, the Disease Systems Biology Program was very productive and co-authored 29 papers. Of these, six have been published in high-impact journals. One of the Program's successes in 2015 was the systems-level analysis of data generated at CPR, for example, the analysis of post-translational modification data to avoid abundance bias in functional annotation (Schölz *et al*., *Nat Methods*, 2015). Furthermore, we received a Big Data grant from the Innovation Fund Denmark, which includes partners from two Danish health regions in addition to companies working in the translational space. This grant focuses on data from millions of patients and will enable us to stratify patients in novel ways according to symptoms and adverse drug reactions.

Technological advances

The Big Data Platform is now established and able to serve both internal needs and external collaborators. We are now in an excellent position to handle data on a huge scale, in the petabyte (1000 terabyte) range. The Platform handles massive amounts of readouts from experiments, as well as subsequent integrative analysis incorporating large external data sets.

Collaborations and international standing

The Program collaborates with many external stakeholders, and my part time employment at Rigshospitalet (Copenhagen) as Medical Informatics Officer also expands the Program's links to the clinical environment. A particular highlight of 2015 was securing funding for the Horizon 2020 project, EU-ToxRisk, which will focus on the interaction between chemicals and protein targets. This project has more than 35 partners around Europe, and includes close collaboration between the Brunak group, Université Paris Diderot, and the University of New Mexico. In addition, I coordinated a white paper on large scale opportunities for precision medicine in Denmark (using whole-genome sequencing and other omics technologies) was published in November 2014.[1]

1. http://www.regioner.dk/aktuelt/nyheder/2015/november/~/media/5DBA6A6343474651B9D58374BF8579BC.ashx.

"We use clinical data to inform the analysis of molecular level data and enable mechanistic insight into disease etiologies for subsequent translational use in the context of precision medicine."

TRANSLATIONAL DISEASE SYSTEMS BIOLOGY

BRUNAK GROUP

Professors
Søren Brunak (Group Leader)

Postdocs
Anders Boeck Jensen
Karina Banasik
Sabrina Gade Ellesøe
Robert Eriksson
Rachita Yadav
Kalliopi Tsafou

PhD Students
Cecilia Engel Thomas
Jessica Xin Hu
David Westergaard
Annelaura Bach Nielsen
Mette Beck
Christian Simon
Freja Hemmingsen Sørup
Isa Kirk
Juan Arranz

Master's and Medical Students
Martin Lademann
Mette Krogh-Pedersen
Marisa Matey Hernandez

Scientific Programers
Troels Siggaard
Andreas Bok Andersen

Exchange Students
Anne Kirstine Band
Alba Gonzalez-Franquesa

EXTERNAL COLLABORATORS

Rudi Westendorp [University of Copenhagen (UCPH), Denmark] aging and public health.

Torben Hansen (UCPH, Denmark) personalized type 2 diabetes treatment.

Oluf Pedersen (UCPH, Denmark) personalized type 2 diabetes treatment.

Karsten Kristiansen (UCPH, Denmark) genome analysis.

Anders Krogh (UCPH, Denmark) genome analysis.

Eske Willerslev (Natural History Museum of Denmark, UCPH, Denmark) human variation.

Thorkild IA Sørensen (UCPH, Denmark) gene–diet interactions, colectomy disease trajectories.

Jens Lundgreen (Rigshospitalet, Copenhagen, Denmark) big biomedical data.

Mikkel H Schierup (Aarhus University, Denmark) bioinformatics.

Thomas Werge (Mental Centre Sct. Hans, Roskilde) network biology and human variation.

Torben Ørntoft (Aarhus University) genomic medicine.

Bernt Guldbrandtsen (Aarhus University) bioinformatics.

Anders Perner (Rigshospitalet, Copenhagen, Denmark) personalized intensive health care.

Henrik Bang Rasmussen (Mental Centre Sct. Hans, Roskilde) personalized medicine and ADHD.

Pierre Baldi (University of California, Irvine, USA) machine learning in bioinformatics.

Ewan Pearson (Dundee, UK) personalized type 2 diabetes treatment.

Paul Franks (Lund University, Sweden) personalized type 2 diabetes treatment.

Tudor I Oprea (University of New Mexico, USA) the druggable proteome.

Pope Moseley (University of New Mexico, USA) disease trajectories.

Leif Groop (Lund University, Sweden) personalized type 2 diabetes treatment.

Bob van de Water (Leiden University, the Netherlands) toxicology.

Lut Overbergh (University of Leuven, Belgium) personalized type 1 diabetes treatment.

Ferran Sanz (University Pompeau Fabra, Spain) medical informatics.

Bernard Thorens (University of Lausanne, Switzerland) personalized type 2 diabetes treatment.

INTERNAL COLLABORATORS

Matthias Mann (Mann Group, Proteomics Program) clinical proteomics and patient stratification.

Lars Juhl Jensen (Jensen Group, Disease Systems Biology Program) medical data mining.

Chunaram Choudhary (Choudhary Group, Proteomics Program) proteomics.

Jesper V Olsen (Olsen Group, Proteomics Program) proteomics and disease systems biology.

2015

Challenges and research aims

The huge diversity of the human proteome is based on a genome that contains less than 20,000 basic protein coding genes. Many of these genes and proteins are likely involved in more than one disease, or in different subphenotypes of a group of diseases that link to a particular organ or tissue. It is, thus, difficult to translate genome and proteome variations to reliably predict the resulting phenotypes.

We address these challenges by using patient-specific healthcare data that contain information on how disease combinations manifest themselves and co-occur in individual patients. Using the Danish personal identification system, we also simultaneously analyze disease co-occurrences in families to help understand how genes and protein complexes may be responsible for the same, or very similar, phenotypes.

The aim is to identify new drug targets, biomarkers, and to provide diagnostic evidence, by: 1) developing data integration tools in supercomputing settings; 2) expanding our already extensive collaborative networks to involve the Capital Region of Denmark and Region Zealand, and clinical partners; and 3) recruiting MD-PhD students who can add clinical and medical knowledge to our team of bioinformaticians, systems biologists, and computer scientists.

STRATEGIC GOALS

- To apply comorbidity healthcare data to understand inherited patterns and correlations between disease subphenotypes, and to combine these with proteome variation data
- To further develop our disease trajectory concept for risk estimation for single diagnoses of, for example, sepsis and the role of diabetes therein
- To facilitate the interpretation of the consequences of human protein kinase variation

Achievements

A key achievement of 2015 was publishing several papers (e.g. Ellesøe *et al.*, *Congenit Heart Dis*, 2015) on congenital heart disease (CHD) subphenotypes and their concordance and discordance in Danish families. Hierarchical cluster analysis of data obtained from patient records (1163 families with CHD in the National Danish Patient Registry, a total of 3080 individuals) showed the familial co-occurrence of six distinct clusters of diagnoses. The discordant co-occurrences largely matched the number of overlapping genes already identified in knockout mice as being associated with the co-occurring pair of subphenotypes (Figure 11). More than a 100 exomes obtained from blood samples were sequenced to understand the distribution of causal variants in protein complex components and many new potential CHD genes were identified.

We also developed a method[2] to facilitate the interpretation of the consequences of human protein kinase variation. Using a kinase-specific random forest approach, we integrated nine methods that predicted the pathogenicity of variants. The variants were visualized in their structural contexts and residues affecting catalytic and drug binding were identified.

Furthermore, we developed our disease trajectory concept by analyzing the temporal order and multiple paths of disease occurrence to estimate the mortality risk of individuals from a 20-year prehistory of a single diagnosis. This work resulted in a paper on sepsis (and the role of different diabetes types therein), and forms the basis for a new big data approach for aggregating long timescales combined with high frequency data from short timescales, e.g., from Intensive Care Units.

Collaborative papers have also been published in *Cell* and *Nature*, including a paper disentangling type 2 diabetes (Forslund *et al.*, *Nature*, 2015) and metformin treatment signatures in the human gut microbiota (Rasmussen *et al.*, *Cell*, 2015).

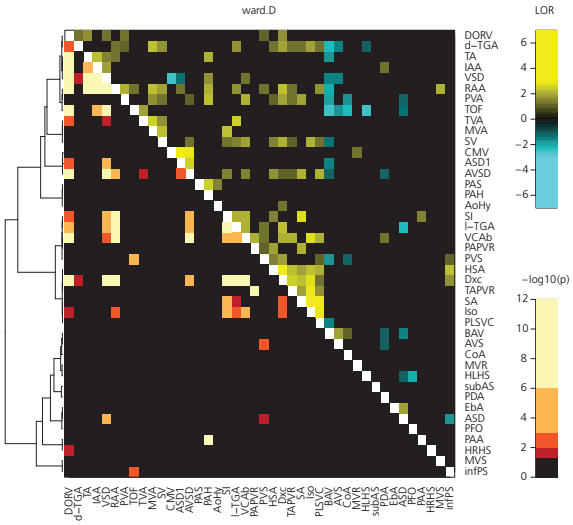


Figure 11 | Hierarchical cluster analysis revealed six distinct clusters of CHD phenotypes (upper part), as well as the number of overlapping genes related to each phenotype–phenotype pair (lower part).

2. The method is now freely available (https://github.com/Rbbt-Workflows/KinMut2).

"We use state-of-the-art data mining and text mining techniques to combine heterogeneous data on a large scale and thereby gain insights into cellular networks."

CELLULAR NETWORK BIOLOGY

JENSEN GROUP

Professor
Lars Juhl Jensen (Group Leader)

Postdoc
David Lyon

PhD Students
Helen Victoria Cook
Oana Palasca
Xiaoyong Pan
Jan Christian Refsgaard
Alberto Santos Delgado
Kalliopi Tsafou

INTERNAL COLLABORATORS

Alicia Lundby (Olsen group, Proteomics Program) proteomics analysis of phosphorylation-dependent binding in cardiac signaling.

Brian Weinert (Choudhary group, Proteomics Program) statistical term-enrichment analysis of proteomics data.

Chunaram Choudhary (Choudhary group, Proteomics Program) proteomics analysis of lysine acetylation.

Michael Lund Nielsen (Nielsen group, Proteomics Program) proteomics analysis of arginine methylation.

Kristina Bennet Emdal (Olsen group, Proteomics Program) proteomics analysis of NGF–TrkA signaling in neuroblastoma.

Chiara Francavilla (Olsen group, Proteomics Program) proteomics analysis of ovarian cancer.

Rosa Jersie-Christensen (Olsen group, Proteomics Program) proteomics analysis of dental calculus from ancient Danish samples.

Louise von Stechow (Olsen group, Proteomics Program) modeling of DNA damage-induced cell cycle regulation.

David Westergaard (Brunak group, Disease Systems Biology Program) knowledge integration and prioritization of drug targets.

EXTERNAL COLLABORATORS

Jan Gorodkin [University of Copenhagen (UCPH), Denmark] data and text mining-based network analysis of non-coding RNAs.

Peer Bork [European Molecular Biology Laboratory (EMBL), Germany] development and maintenance of the STRING, eggNOG, STITCH, and SIDER web resources.

Christian von Mering (University of Zurich, Switzerland) development and maintenance of the STRING, eggnog, and STITCH web resources.

Michael Kuhn (University of Cologne, Germany) development and maintenance of the STRING, eggNOG, STITCH, and SIDER web resources.

Evangelos Pafilis (Hellenic Center for Marine Research, Greece) development of the ENVIRONMENTS and EXTRACT text mining tools.

Enrico Cappellini (UCPH, Denmark) proteomics analysis of dental calculus from ancient Danish samples.

Sean I O'Donoghue (Commonwealth Scientific and Industrial Research Organisation and Garvan Institute of Medical Research, Australia) scientific visualization of localization and expression data.

Tudor Oprea (University of New Mexico, USA) knowledge integration and prioritization of drug targets.

Yesid Cuesta Astroz (Universidade Federal de Minas Gerais, Brazil) protein network analysis of host–pathogen interactions.

John "Scooter" Morris (University of California, San Fransisco, USA) integration of the Cytoscape network tool with the STRING resource.

Burkhard Rost (Technische Universität, Munich, Germany) application of text mining to protein subcellular localization annotation.

2015

Challenges and research aims

High-throughput technologies continue to revolutionize the way we study biological systems. A few decades ago, the only option was to study proteins and other biomolecules one at a time. We are getting ever closer to being able to simultaneously study all proteins and transcripts in a human cell. However, this ability to rapidly produce vast quantities of data brings with it new challenges. How can we efficiently analyze large datasets, integrate heterogeneous datasets, visualize the results, and compare them with the published literature?

These are the types of challenges that our group and collaborators aim to tackle. We work closely with the groups in the Proteomics Program with the goal of developing better statistical methods for the analysis of mass spectrometry-based investigations of post-translational modifications. We are developing a suite of community databases and web resources that aims to systematically bring together high-throughput datasets and, through the use of in-house text mining algorithms, information from the published literature. Finally, we work with visualization experts to present the results (for example, see Figure 12).

Our group is very well placed to address these challenges, owing to the know-how and infrastructure developed over many years within biomedical data mining in general, and particularly in text mining. This is further supported by an exceptionally strong international network of collaborators with diverse scientific backgrounds.

STRATEGIC GOALS

- To release user-friendly databases and associated web resources that unify manual annotations, automatic text mining, and high-throughput data on protein–protein, protein–tissue, and protein–disease associations
- To apply existing in-house computational methods to new mass spectrometry-based proteomics studies of cellular signaling in childhood cancers

Achievements

A key achievement this year was the launch and publication of STRING version 10 in *Nucleic Acids Research* (Szklarczyk *et al.*, *Nucleic Acids Res*, 2015), which was also presented by Lars J Jensen in a plenary session at the Keystone Symposia Conference 'The Human Proteome.' STRING is a database of functional associations between proteins that we develop and maintain in close collaboration with research groups in Germany and Switzerland. It is used by thousands of scientists around the world every day, and earlier publications have received more than 1500 new citations in the past 2 years alone.

In 2015, we developed several other resources that provide a solid foundation for future research activities. This includes the publication of new integrative web resources on the disease associations (Pletscher-Frankild *et al.*, *Methods*, 2015) and tissue expression (Santos Delgado *et al.*, *PeerJ*, 2015) of proteins. The TISSUES web resource (see Figure 12) provides an at-a-glance overview of evidence from database annotations, from proteomics and transcriptomics studies as well as from automatic text mining of the scientific literature. The web resources that we have published have formed the basis for our work within the large National Institutes of Health (USA) program 'Illuminating the Druggable Genome,' which aims to shed light on the lesser studied proteins that can be targeted by drugs. We also developed a new statistical method in close collaboration with the Choudhary group (Proteomics Program), which improves enrichment analyses of proteomics experiments, and was published in *Nature Methods* (Schölz *et al.*, *Nat Methods*, 2015).

Furthermore, we have continued our fruitful collaboration with the Proteomics Program on the analysis of post-translational modifications, which has resulted in several joint publications including papers in *Nature Biotechnology* and *Science Signaling* (Emdal *et al.*, *Sci Signal*, 2015; Schölz *et al.*, *Nat Biotechnol*, 2015).

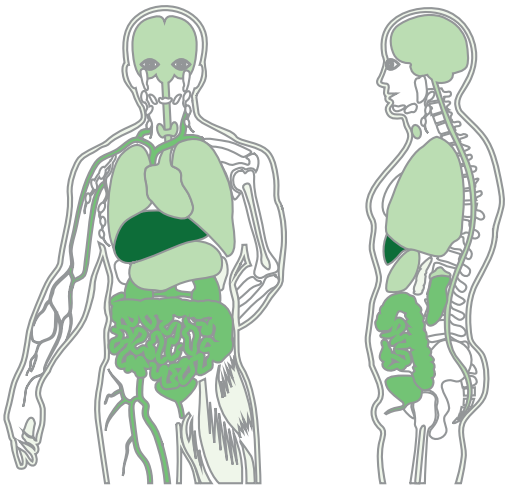


Figure 12 | The TISSUES web resource allows users to easily obtain a color-coded schematic of the tissue expression of any human protein of interest. Here, results for a pulmonary surfactant protein, SFTPD, are shown.

BIG DATA MANAGMENT PLATFORM

Professor
Lars Juhl Jensen (Platform Leader)

Computer Specialist
Rebeca Quinones

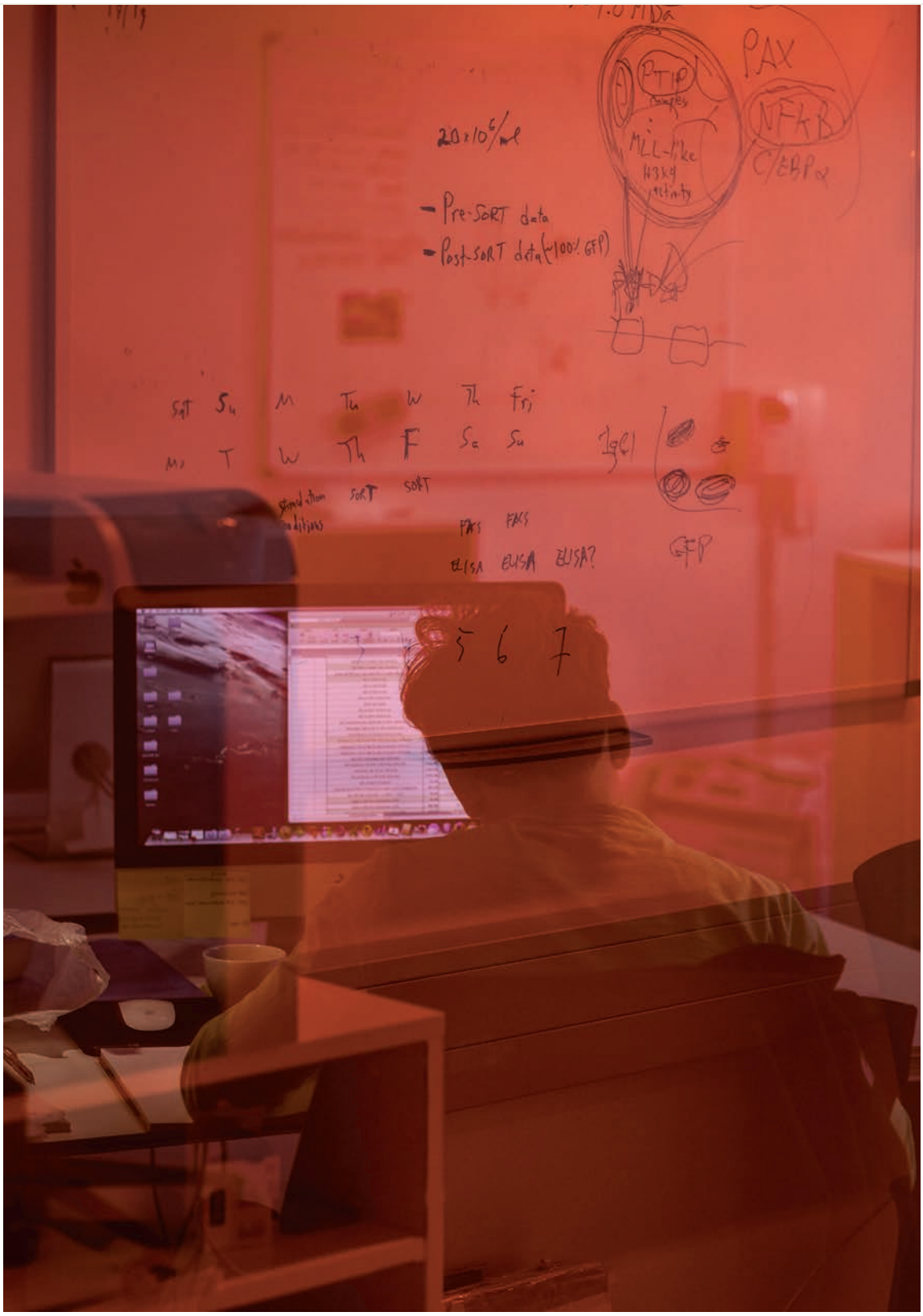
The purpose of the Big Data Management Platform is to provide a shared, scalable computational infrastructure to handle the vast amounts of data produced by the various technology Platforms at CPR, such as raw mass spectrometry and imaging data. Therefore, the primary users are the other Platforms, which need stable data management solutions, rather than individual researchers at CPR.

The Platform currently provides large-scale storage and backup solutions to safely capture and archive data. In the future, the Platform will also be able to provide the computational power required for the initial analysis of data, which is often very computer-intensive. So far, storage and backup has been provided via existing infrastructure at the Center for Biological Sequence Analysis at the Technical University of Denmark (TUD).

In 2015, the Platform purchased a scalable multi-tier storage system from Oracle which, at the time of writing, is just about to be delivered. The new storage system has 200 terabytes (TB) of hard storage, 2 × 450 TB of storage in the form of tape robots, and practically unlimited archival storage in the form of offline tapes.

To provide the necessary computer power, the Platform has access to the new National Life Science Supercomputer: Computerome (Figure 13). As of June 2015, Computerome ranks as the 161st fastest supercomputer in the world, and is a joint project between UCPH, the TUD and the Danish e-infrastructure collaboration. Computerome will have direct access to the new storage system, allowing for convenient big data analysis of, for example, raw mass spectrometry data.

Figure 13 | National Life Science Supercomputer: Computerome.


PAX
NFkB
C/EBPα
MLL-like
H3K4
activity
20x10⁶/ml
- Pre-SORT data
- Post-SORT data(+100% GFP)
SORT
SORT
FACS
FACS
ELISA
ELISA
ELISA?
GFP

PROTEIN SIGNALING PROGRAM

Chromatin Structure and Function
DANIEL GROUP

Chromosome Stability and Dynamics
LUKAS GROUP

Ubiquitin Signaling
MAILAND GROUP

Mitotic Mechanisms and Regulation
NILSSON GROUP

Protein Imaging Platform

FROM THE PROGRAM DIRECTOR
JIRI LUKAS

"Our overall aim is to increase our mechanistic understanding of how protein pathways are wired to signaling networks and how these coordinate complex biological processes."

The overarching role of the Protein Signaling Program is to provide a strong biomedical framework, with which we make full use of our state-of-the-art protein technologies to elucidate protein-driven mechanisms involved in complex biological processes. We focus on proteins that maintain genome stability because of their fundamental role in many pathological conditions, including cancer, metabolic disorders, and aging, hematopoietic and immune deficiencies.

Organization

The four groups in this Program bring together broad expertise in protein signaling, spanning ubiquitin regulation (Niels Mailand), chromosome segregation (Jakob Nilsson), mouse models for DNA repair deficiencies (Jeremy Daniel), and chromatin responses to genotoxic stress (Jiri Lukas).

Technological advances

The Program's success relies strongly on synergistic scientific interactions with other Programs and active contributions to CPR's technological Platform concept. Scientists from all the program groups closely interact with the Protein Imaging Platform, not just as users but as active contributors, to improve and develop the analytical possibilities of our microscopes and the power of advanced image analysis. A significant technological achievement is the introduction of the Quantitative Image-Based Cytometry technique, originally developed by Luis Toledo (Lukas group), which has transformed dynamic cell-based studies by enabling unbiased, high-content, quantitative, and simultaneous imaging of proteins in large cell populations. Another significant development is the establishment of the joint Flow Cytometry Facility with the neighboring DanStem center. CPR's activities in this area are supervised by Jeremy Daniel.

Research highlights

In 2015, all groups made important discoveries, in their respective areas, in collaboration with other CPR Programs. Niels Mailand's group identified and characterized SLF1 and SLF2 as novel regulators of DNA interstrand crosslink repair (Räschle *et al*., *Science*, 2015). This highly significant discovery was integrated into a large proteomic endeavor coordinated by Matthias Mann (Proteomics Program), which has resulted in an unmatched catalogue of proteins involved in an entire DNA repair pathway. The Mailand group has, in close collaboration with the Choudhary group (Proteomics Program), discovered ubiquitylated histone H1 as a long-sought missing link in a mechanism that coordinates chromatin responses to DNA damage (Thorslund *et al*., *Nature*, 2015). In addition, I teamed up with Michael L Nielsen's group (Proteomics Program) and the Protein Production and Characterization Platform to uncover the role of poly(ADP-ribose) and the unstructured proteome in resolving some of the earliest molecular events at damaged chromosomes (Altmeyer *et al*., *Nat Commun*, 2015)]. Jakob Nilsson's and Jeremy Daniel's groups also contributed significantly to the Program's scientific output by elucidating the role of the kinetochore-associated proteins BubR1 and RZZ in safeguarding the fidelity of chromosome segregation (Zhang *et al*., *Nat Commun*, 2015), and by elucidating the crosstalk between histone methylation and transcription during antibody gene diversification (Starnes *et al*., *Genes Dev*, in press), respectively. These discoveries also strongly benefited from CPR synergy, involving groups from the Proteomics and Protein Structure and Function Programs.

Collaborations and international standing

The program's groups continued to develop their national and international standing in 2015 by collaborating with other leading research groups, attracting competitive international funding, and receiving prestigious awards. A few highlights: Niels Mailand received the prestigious EliteForsk prize (Danish National Research Foundation); Jiri Lukas gave the prestigious Curie Lecture in Paris, France and the Ernst Caspari Lecture in Gottingen, Germany, and organized the Keystone Symposium 'Genomic Instability and DNA repair' in Whistler, Canada; Jakob Nilsson organized the European Molecular Biology Organization (EMBO) Workshop 'Dynamic Kinetochore' in Denmark.

"We combine mouse genetics with proteomic and imaging expertise at CPR to gain deeper understanding of how DNA damage impacts the development and aging of our immune system."

CHROMATIN STRUCTURE AND FUNCTION

DANIEL GROUP

Associate Professor
Jeremy A Daniel (Group Leader)

Postdocs
Andreas Mund
Ewa Ohlsson
Linda Starnes
Dan Su
Valentyn Oksenych

PhD Student
Laura Pikkupeura

Technicians
Martina Kubec
Rebeca Soria

Laboratory Assistant
Christian Buch

INTERNAL COLLABORATORS

Michael Lund Nielsen (Nielsen group, Proteomics Program) identifying functional proteins at DNA breaks in primary lymphocytes using proteomics.

Chunaram Choudhary (Choudhary group, Proteomics Program) elucidating mechanisms of PTIP-MLL3/MLL4 methyltransferase complex function in B lymphocytes.

Niels Mailand (Mailand group, Protein Signaling Program) elucidating functional roles of DNA damage response proteins in mice.

Guillermo Montoya (Montoya group, Protein Structure and Function Program) the mechanism of how the PTIP/PA1 complex functions in transcription.

Jiri Lukas (Lukas group, Protein Signaling Program) dissecting mechanisms of genome stability maintenance using high-content imaging.

EXTERNAL COLLABORATORS

Andres Lopez-Contreras (University of Copenhagen (UCPH), Denmark) elucidating the role of replicative stress in immune cells.

Joan Yuan (Lund University, Sweden) studying the functional differences between fetal and adult immune cells.

Andre Nussenzweig (National Cancer Institute/ National Institutes of Health (NCI/NIH), USA) elucidating the functions of the PTIP-MLL3/MLL4 methyltransferase complex in DNA repair and recombination.

Francesca Cole (MD Anderson/University of Texas, USA) the roles of DNA damage response proteins during germ cell development.

Sharon Dent (MD Anderson/University of Texas, USA) the roles of lysine acetylation in B lymphocytes.

Tanya Paull (University of Texas, USA) the mechanism of ATM activation.

2015

Challenges and research aims

The impact that various DNA damage stresses have on the development and aging of our immune system remains incompletely understood. This challenge is the main priority of our group because aberrant DNA repair in stem, progenitor, and mature blood cells can lead to immunodeficiency, cancer, and other age-related pathologies. In particular, although the accumulation of DNA damage is observed in blood stem cells of mice and humans as individuals age, the source of this genotoxic stress is still unclear. Moreover, many multi-functional proteins and protein complexes participate in programmed DNA damage that generates antibody diversity in lymphocytes, yet our understanding of how transcription, DNA damage, and subsequent repair of DNA breaks coordinately occurs at antibody genes is rudimentary at best. Our group is well placed to tackle these challenges, with our expertise in generating and characterizing mouse genetic models with transgenic or deficient expression of DNA damage response caretakers of the genome, in collaboration with the Transgenic Core Facility at the University of Copenhagen (UCPH). Moreover, we also apply our in-depth knowledge of immunoglobulin heavy-chain class-switch recombination (Linda Starnes and Dan Su) and cytogenetic and DNA damage foci analyses (Andreas Mund, together with the Protein Imaging Platform).

We have strengthened our collaboration with Andres Lopez-Contreras at UCPH this year, which has allowed us to pool our knowledge and resources to investigate whether mouse models with reduced replication-associated DNA damage (so-called 'replicative stress') have a healthier blood system than those with normal levels of replicative stress. The long-term aim of this project is to provide insight into the development of treatments for age-related diseases of the blood system.

STRATEGIC GOALS

- To develop and apply a label-free quantitative proteomic method for identifying chromatin-associated proteins at DNA breaks in lymphocytes
- To elucidate how the PTIP-MLL3/MLL4 methyltransferase complex coordinates programmed DNA damage in B lymphocytes
- To characterize the impact of stress from replication-associated DNA damage on hematopoietic stem cells

Achievements

A key achievement this year was writing and submitting the first 'home-grown' manuscript from our group, which was accepted for publication in *Genes & Development* (Starnes *et al.*, *Genes Dev*, in press). In this study, we discovered an unexpected function for a subcomplex, consisting of PTIP and PA1 proteins, in promoting the transcription of certain types of antibodies through a mechanism independent from its well-known association with the MLL3/MLL4 multisubunit histone methyltransferase complex (see Figure 14). Antibody class-switching is crucial for the generation of certain types of antibodies, such as IgG, by B cells that can then help to clear infections in the body. In the study, we performed quantitative interaction proteomics, led by Linda Starnes in collaboration with Chunaram Choudhary (Proteomics Program), and verified predictions made in PA1- and MLL3/MLL4-deficient mouse models. As well as identifying PA1 as a novel antibody class-switching factor, we showed a specific function for the PTIP/PA1 subcomplex for the first time, and opened up further avenues of investigation to study the relationship between histone methylation and transcription during antibody gene diversification. These findings may, thus, have important implications for the design of treatments for antibody-driven pathologies, such as hypogammaglobulinemia or some autoimmune diseases.

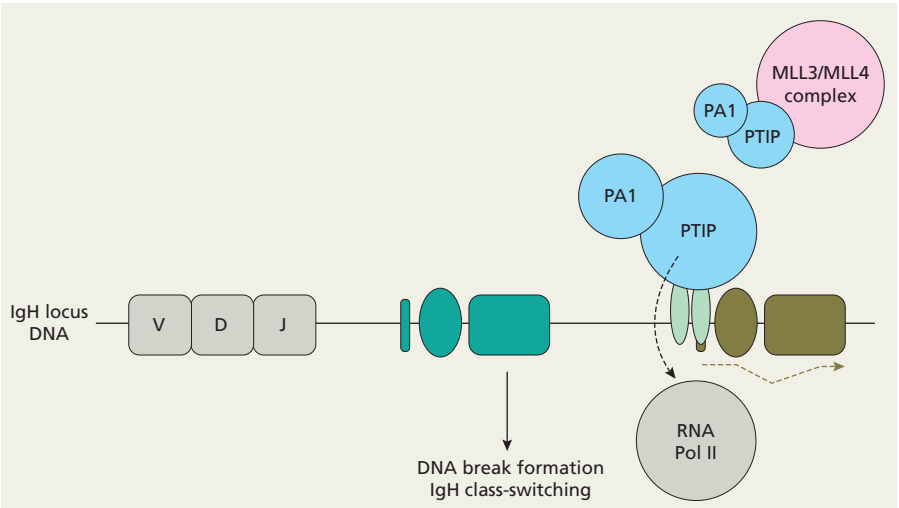


Figure 14 | Model of how a stable PTIP-PA1 subcomplex promotes programmed DNA damage for antibody class-switching independently from its known association with the MLL3/MLL4 complex.

"Utilizing quantitative protein imaging of large cell populations, we aim to understand physiological thresholds of DNA repair modules to explain the genesis of diseases caused by unstable genomes."

CHROMOSOME STABILITY AND DYNAMICS

LUKAS GROUP

Professors
Jiri Lukas (Group Leader)
Claudia Lukas (Senior Scientist and Protein Imaging Platform Leader)

Postdocs
Luis Toledo
Kai Neelsen
Kumar Somyajit
Ronni Sølvhøj Pedersen

PhD Student
Fena Ochs
Master's Student
Emilie Baron

Technicians
Maj-Britt Druedahl Rask
Merete Grøfte

Laboratory Assistants
Andreas Willems
Philip Becher Jørgensen

INTERNAL COLLABORATORS

Stephanie Jungmichel, **Michael L Nielsen** (Nielsen Group, Proteomics Program) and **Irina Pozdnyakova**, **Werner Streicher** (Protein Production and Characterization Platform) the role of poly(ADP-ribose) and the intrinsically unstructured proteome in orchestrating early stages of DNA repair.

Stephanie Munk and **Jesper V Olsen** (Olsen group, Proteomics Program) the role of protein phosphorylation in genome maintenance after DNA replication stress and chromosome breakage.

Andreas Mund and **Jeremy Daniel** (Daniel group, Protein Signaling Program) chromatin regulation upon DNA replication stress.

Rajat Gupta and **Chunaram Choudhary** (Choudhary group, Proteomics Program) phenotypic classification of the uncharacterized fraction of the human genome.

Stefano Stella and **Guillermo Montoya** (Montoya group, Protein Structure and Function Program) fluorescence tagging of endogenous genome caretakers using gene-editing technology.

EXTERNAL COLLABORATORS

Andre Nussenzweig (NCI/NIH, USA) ubiquitin-mediated signaling pathways involved in DNA repair.

Jan Ellenberg, **Rainer Pepperkok**, **Beate Neumann**, **Jean-Karim Heriche** (European Molecular Biology Laboratory, Heidelberg, Germany) phenotypic classification of the uncharacterized fraction of the human genome.

Daniel Gerlich (Institute of Molecular Biotechnology, Vienna, Austria) consequences of mitotic perturbations for genome integrity.

Ian Hickson [Center for Chromosome Stability, University of Copenhagen (UCPH), Denmark] the role of recombination-mediated DNA repair in genome integrity maintenance.

Jiri Bartek (Danish Cancer Society, Copenhagen, Denmark) the molecular pathology of cellular responses to DNA damage.

2015

Challenges and research aims

We are interested in how proteins that guard the integrity of the human genome assemble into functional pathways, and how these pathways organize themselves in the three-dimensional space of the cell nucleus. In our view, the genome integrity field has now reached the stage when we need to ask not only 'how does DNA repair work?', but also 'what are the physiological limits of the underlying biochemical reactions?' A specific outstanding question is 'how much damage can a cell endure, while still being able to choose a repair pathway that will guard against cancer-predisposing mutations and also shield healthy parts of the genome from untimely or excessive DNA and chromatin transactions?' Our group is ideally positioned to address these challenges, thanks to our long-standing innovative approaches to visualize cellular responses to genotoxic stress in their physiological environment (i.e., the nucleus of a living cell).

Achievements

The key achievement this year was the discovery that the anionic biopolymer poly(ADP-ribose) (PAR) is one of a growing list of molecules that organize the subcellular architecture of damaged chromatin. Specifically, we found that by virtue of its charge, PAR can trap intrinsically disordered proteins and trigger their dynamic assembly into higher-order structures by means of a liquid–liquid phase separation. We showed that this mode of regulation operates on damaged chromosomes very early after DNA breakage (see Figure 15), and that the PAR-seeded phase separation of unstructured proteins provides cells with an opportunity to filter protein interactions, thereby contributing to repair pathway choice. This study (Altmeyer *et al.*, *Nat Commun*, 2015) was carried out in close collaboration with Stephanie Jungmichel and Michael Lund Nielsen (Proteomics Program), and Irina Pozdnyakova and Werner Streicher (Protein Production and Characterization Platform), and the paper was highlighted in the news section of *Nature Structural and Molecular Biology* [*Nat Struct Mol Biol*. **22**, p. 655, (2015)].

During 2015, we also further advanced our Quantitative Image-Based Cytometry (QIBC) high-content imaging technique, which was originally developed by Luis Toledo in our lab to study cellular limits to DNA replication stress. With the latest refinements, this technique enables us to interrogate DNA double-strand break (DSB) repair pathways in real time and at the cell population level. This puts us in a strong position to investigate how cells select, from many available repair modules, those that allow the repair of highly toxic DNA lesions with the highest possible fidelity. A dedicated project on this subject is in progress by Fena Ochs, Kumar Somyajit, and Claudia Lukas, and we look forward to the conceptual insights and technological advances made by this research.

Finally, we would like to highlight that, after a very successful PhD project at the Indian Institute of Science (Bangalore, India), Kumar Somyajit joined our group in May 2015. He brings to the lab his original conceptual thinking and a wealth of complementary skills in state-of-the-art biochemical assays such as iPOND, DNA combing, DNA repair reporter assays, and many others. Kumar's major focus in our group is how human cells protect their replicating genomes against destabilizing intrinsic and environmental assaults.

STRATEGIC GOALS

- To define the role of the unstructured proteome in cellular responses to DNA damage
- To further develop high-content protein imaging to a level that enables quantitative monitoring of DNA repair pathway choices in large cell populations

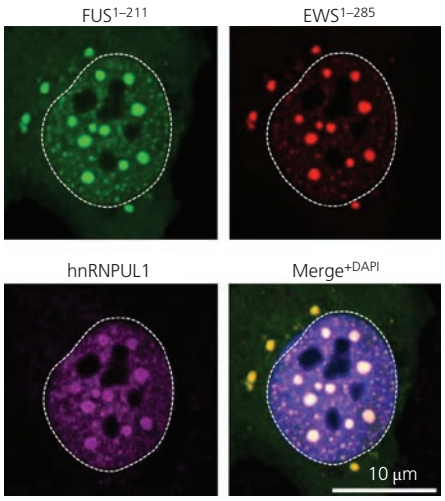


Figure 15 | Intrinsically unstructured proteins (labeled with different colors) undergo liquid–liquid demixing seeded by poly(ADP-ribose).

"Drawing on the expertise in proteomics and advanced microscopy within CPR and externally, we are discovering important new proteins and signaling processes that protect genome stability after DNA damage."

UBIQUITIN SIGNALING
MAILAND GROUP

Professor
Niels Mailand (Group Leader)

Associate Professors
Simon Bekker-Jensen
Tina Thorslund

Postdocs
Godelieve Smeenk
Petra Schwertman
Claire Guerillon
Sara Lund Poulsen
Wenjing Zheng
Yasuyosi Oka

PhD Students
Saskia Hoffmann
Rebecca Kring Hansen
Maxim Tollenaere
Anita Ripplinger
Peter Haahr
Bine Hare Villumsen
Stine Smedegaard
Divya Achuthankutty

Master's Student
Louise Nilausen

Laboratory Assistant
Julie Nielsen
Greta Tuckute

INTERNAL COLLABORATORS

Chunaram Choudhary (Choudhary group, Proteomics Program) proteomic analysis of ubiquitin-dependent signaling in the DNA damage response.

Jeremy Daniel (Daniel group, Protein Signaling Program) mouse knockout models of proteins involved in the response to DNA DSBs.

Guillermo Montoya (Montoya group, Protein Structure and Function Program) structural and biophysical characterization of new factors in the DNA damage response.

EXTERNAL COLLABORATORS

Matthias Mann (Max Planck Institute of Biochemistry, Munich, Germany) mass spectrometry-based mapping of proteins acting in the context of damaged DNA.

Titia Sixma (Netherlands Cancer Institute, Amsterdam, the Netherlands) biochemical studies of histone ubiquitylation.

Anja Groth [Biotech Research and Innovation Centre, University of Copenhagen (UCPH), Denmark] studies of stressed replication forks and their composition.

Andres Lopez-Contreras (Center for Chromosome Stability, UCPH, Denmark) mouse models of new factors involved in responses to replication stress.

Ian Hickson and **Hocine Mankouri** (Center for Chromosome Stability, UCPH, Denmark) development of new methods for inducing and studying site-specific replication blocks in human cells.

2015

Challenges and research aims

We aim to understand the cellular signaling processes that underpin and regulate the DNA damage response (DDR), a sophisticated network of pathways that re-establish the integrity of the genome after genotoxic insults and have a central role in safeguarding cell and organism fitness. In particular, we have only a partial understanding of the numerous signaling processes within the DDR, their wiring, and coordination, as well as their biological ramifications. Hence, there is a need for new innovative approaches capable of defining the scope of cellular processes that respond to DNA damage in a systematic and unbiased manner.

Using powerful proteomics and advanced cell imaging technologies, together with our experience in characterizing cellular signaling processes, we are well placed to identify new factors and functionally dissect the mechanisms that operate in cells to protect genome stability. We have also begun to analyze the biological functions of new DDR factors using mouse knockout models and structural biology approaches, via in-house and local collaborations.

STRATEGIC GOALS

- To comprehensively identify the cellular proteins acting in the context of damaged DNA and to mechanistically define their functions in DNA repair and genome stability maintenance
- To characterize how cellular signaling processes mediated by ubiquitin and related small modifier proteins orchestrate and regulate cellular responses to DNA damage

Achievements

In 2015, we published a range of papers in high-impact journals on important new factors and mechanisms underlying the DNA damage response in human cells. These achievements were made possible by collaborations with world-leading proteomics experts at CPR and beyond. One key achievement this year was our discovery, published in *Nature* (Thorslund *et al*., *Nature*, 2015) and highlighted in *Nature Reviews Molecular Cell Biology* [*Nature Rev Mol Cell Biol*. **16**, p. 700 (2015)], that ubiquitin-dependent modification of H1-type linker histones has a central, but hitherto overlooked, role in promoting the cellular response to DNA DSBs, a particularly cytotoxic DNA lesion (see Figure 16). This important finding, made in collaboration with Chunaram Choudhary's group (Proteomics Program), not only solves a major outstanding question about how ubiquitin-dependent signaling promotes the cellular response to DSBs, but also suggests for the first time that, along with the four core histones, histone H1 has an integrated role in the 'histone code' for DNA repair and potentially many other DNA-associated processes.We will devote extensive efforts to study this emerging function of histone H1 in the coming years by devoting extensive efforts to study this emerging function of histone H1 and its post-translational modifications in the coming years, we hope to obtain fundamental new insights into how cells protect the integrity of their genomes

A second highlight was our collaboration with Matthias Mann (Proteomics Program and Max Planck Institute of Biochemistry, Munich), with whom we developed a powerful new proteomics-based method termed CHROMASS, which allows systematic mapping of the dynamic protein landscape at sites of DNA damage for the first time. Using this method, we comprehensively defined the inventory of cellular proteins that function at DNA interstrand crosslinks, a type of DNA lesion induced by many chemotherapeutic agents. From these surveys, we identified two new proteins, which we termed SLF1 and SLF2, which recruit the SMC5/6 cohesion complex to damaged DNA (Räschle *et al*., *Science*, 2015). In addition to SLF1 and SLF2, the CHROMASS datasets revealed a range of other new factors present at sites of DNA damage, many of which we are currently characterizing in the lab. We are also applying CHROMASS to survey the composition of chromatin containing other types of DNA lesions. Collectively, we are confident that these studies will enable us to identify and characterize additional new factors that protect genome stability after DNA damage in the years ahead.

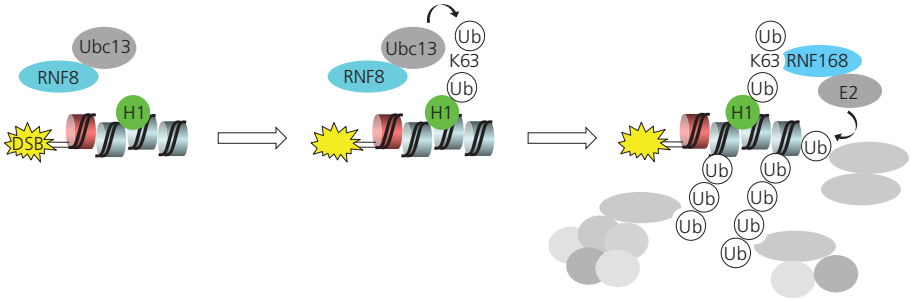


Figure 16 | Ubiquitylation of histone H1 at DNA DSBs initiates a recruitment program leading to accumulation of multiple DNA repair factors near the lesions. From (Thorslund *et al*., *Nature* 2015).

"Our ability to integrate biochemistry and cell biology has allowed us to uncover fundamentally new aspects of mitotic regulation that are conserved throughout eukaryotes."

MITOTIC MECHANISMS AND REGULATION

NILSSON GROUP

Associate Professor
Jakob Nilsson (Group Leader)

Postdocs
Daniel Geoffrey Hayward
Gang Zhang
Garry Gray Sedgwick
Julie Schou
Marie Sofie Yoo Larsen
Thomas Kruse

PhD Students
Emil Peter Thrane Hertz
Jamin Benjamin Hein

Master's Students
Dimitriya Hristoforova Garvanska
Natalie Kim

Laboratory Assistants
Dimitriya Hristoforova Garvanska
Marie Winther-Sørensen

INTERNAL COLLABORATORS

Chunaram Choudhary (Choudhary group, Proteomics Program) genetic analysis of the mitotic degradation machinery.

Jesper V Olsen (Olsen group, Proteomics Program) elucidation of protein phosphatase regulation in major signaling pathways.

Michael Lund Nielsen (Nielsen group, Proteomics Program) interaction of checkpoint proteins using proximity dependent ligation techniques.

Guillermo Montoya (Montoya group, Protein Structure and Function Program) structural aspects of protein phosphatases.

EXTERNAL COLLABORATORS

Jean-Paul Vincent (The Francis Crick Institute, London, UK) role of protein phosphatases in Wnt signaling.

Norman Davey (University College Dublin, Ireland) bioinformatic identification of protein phosphatase interactors.

2015

Challenges and research aims

In-depth understanding of how our genetic material is safely and accurately passed on through the generations is vital as it is a prerequisite for life. The accurate partitioning of genetic material to two new daughter cells during cell division is a particularly vulnerable part of the process, since failure to accurately complete this step has severe consequences for human health. For this reason, my group focuses on elucidating the molecular details of important cellular mechanisms that safeguard genetic material. A major unresolved question is how small signaling platforms on the genetic material, the kinetochores, are able to control events throughout the cell by the generation of diffusible inhibitors. We are in a unique position to tackle this problem because of our expertise in sophisticated live cell imaging approaches that we integrate with the Mass Spectrometry and Protein Production and Characterization Platforms at CPR. The integration of these techniques and the collaborative environment at CPR is crucial to our success.

STRATEGIC GOALS

- To continue to discover how protein phosphatases regulate cellular signaling by identifying consensus motifs of interaction and, specifically, disrupt these in important disease relevant pathways
- To define the role of mitotic checkpoint proteins in regulating chromosome segregation

Achievements

The key achievement for us this year was the discovery by Gang Zhang of how two key components of the mitotic checkpoint, BubR1 and RZZ, localize to kinetochores to control chromosome segregation (see Figure 17). A striking outcome of this work was the realization that BubR1 and RZZ antagonize each other locally to make mitotic checkpoint signaling responsive to small perturbations. The work was published in (Zhang *et al*., *Nat Commun*, 2015) and further discussed in an editorial in *Cell Cycle* (Nilsson *et al*., *Cell Cycle*, 2015), and forms an important foundation for our long-term goal to understand how kinetochores control cell division. Follow-up work by Gang Zhang is ongoing and is supported by a grant from the Danish Council for Independent Research. An additional outcome of this work has been the development of small peptides by Gang Zhang that precisely interfere with the localization of BubR1 to kinetochores and arrest cells in mitosis. We are currently conducting tissue culture experiments to investigate whether such peptides can specifically target cancer cells that have an aberrant number of whole chromosomes.

To increase the visibility of our research and foster interactions internationally, we organized an EMBO Workshop in Copenhagen on the 'Dynamic Kinetochore,' which was sponsored by competitive grants from EMBO and the Carlsberg Foundation. In total, 90 people from around the world, working on different aspects of cell division, gathered for the workshop.

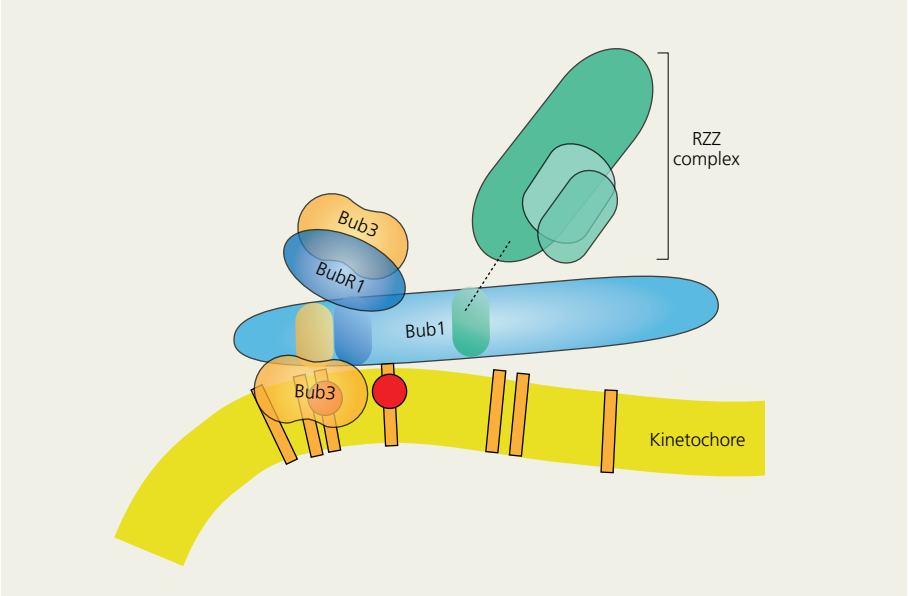


Figure 17 | Coordination of BubR1 and RZZ localization to kinetochores by Bub1.

PROTEIN IMAGING PLATFORM

Professor
Claudia Lukas (Platform Leader)

Microscopy Specialist
Jutta Bulkescher

Image Analysis Specialist
Gopal Karemore

Entering into its second year of existence in 2015, the Protein Imaging Platform has seen major developments to further its provision of extended support and services to an increasing number of microscopy users at CPR.

First, we have procured an additional high-throughput screening microscope and computer workstation for offline image analysis in order to expand the Platform's capacity for this technology, which is in high demand. Second, thanks to great support from CPR's Big Data Management Platform, we have secured large volume storage and backup of microscopy data together with easy desktop computer access to a new, central data storage device. Finally, we have set up a new cell culture laboratory, enabling microscopy users to prepare their cells for live imaging or high-content screening in close proximity to the microscopes. This also houses a new cell sorter, a key addition to the Flow Cytometry Facility initiated by Jeremy Daniel (Protein Signaling Program) in collaboration with Gelo dela Cruz (Flow Cytometry Core Facility, DanStem).

Importantly, hands-on support, which is provided by microscopy specialist Jutta Bulkescher for a variety of different microscopes, is now complemented by support for image analysis by computer scientist Gopal Karemore, who joined the Platform at the end of 2014. This year, Gopal has also been developing custom software tools for image analysis and providing assistance with data visualization and statistical analysis. Our approach of attaching equal importance to image acquisition and image analysis sets us apart from what many other imaging facilities provide, by extending our imaging pipeline to the final quantitative data analysis step (see Figure 18).

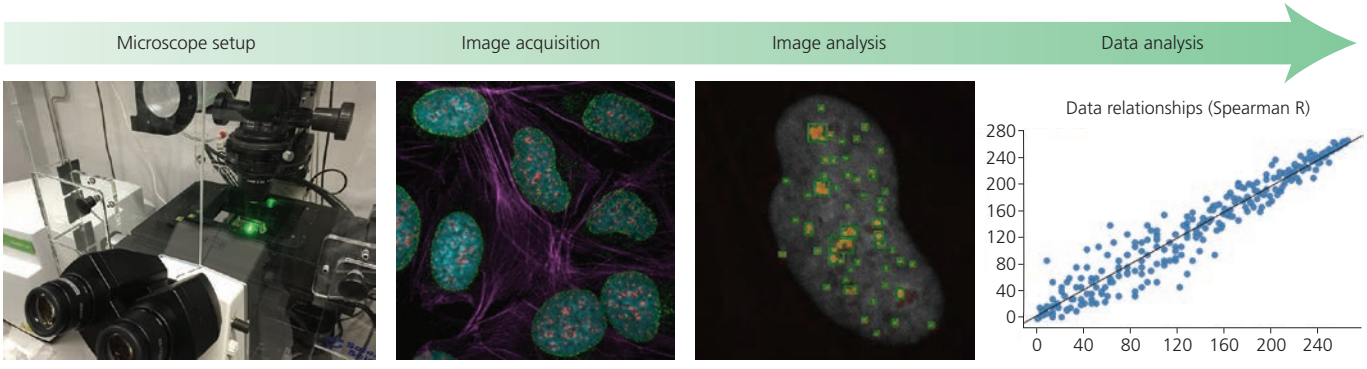


Figure 18 | The complete pipeline for microscopy support provided by the Protein Imaging Platform.

The Protein Imaging Platform actively networks with other microscope facilities in Denmark (e.g., the Core Facility for Integrated Microscopy, the University of Copenhagen) and in Europe (e.g., the European Molecular Biology Laboratory), and shares imaging support with the DanStem Novo Nordisk Foundation Research Center based on reciprocal two-way collaboration. We also welcome external microscopy users from Copenhagen and beyond to our regular seminar activities and tutorials.

PROTEIN STRUCTURE AND FUNCTION PROGRAM

Macromolecular Crystallography
MONTOYA GROUP

Protein Function and Interactions
WIKSTRÖM GROUP

Protein Production and Characterization Platform

FROM THE PROGRAM DIRECTOR
GUILLERMO MONTOYA

"Our main objective is to expand the mechanistic understanding of key cellular processes in cell cycle progression and genome integrity."

Macromolecules underlie all biological processes and either play dynamic roles in catalysis or signaling, or have static roles in scaffolding or information storage. Assemblies of biomolecules and their carefully coordinated interactions carry out nearly every major process in a cell. Malfunctions in protein pathways that orchestrate cell proliferation and guard the integrity of the genome are involved in many diseases. However, the functions of these proteins cannot be understood if we consider them individually and separate from their molecular and cellular contexts. There is therefore a great need to extend our limited comprehension of the cellular organization, localization, and actions of these molecular machines.

Organizational development

In 2015 we continued organizing the Program, and we are now well placed to improve our understanding of biomolecules by combining biochemical, biophysical, structural, cellular, and computational approaches. For maximum effectiveness, we synergize strongly with other research groups at CPR, at the University of Copenhagen (UCPH), and at an international level.

Group Leader Mats Wikström has recently accepted a new position in the pharmaceutical industry and we wish him well in this next step of his career. Members of his group have either moved to biotech companies in the Öresund region or have been relocated inside the Program. We expect to open new Group Leader positions and to recruit new Junior Group Leaders in our Program, to complement our new efforts in electron microscopy.

Electron microscopy enables us to study structures with atomic resolution, which is an essential prerequisite for the development of novel antibiotics and drugs. We are therefore starting to expand some of our projects using this technology, which is highly complementary with X-ray crystallography.

Research highlights

In total, the Program published 12 papers in 2015, four of them in high profile journals such as *Nature Structural and Molecular Biology* (Molina *et al*., *Nat Struct Mol Biol*, 2015), *Nature Communications* (De Biasio *et al*., *Nat Commun*, 2015), *Journal of Cell Biology* (Hoffmann *et al*., *J Cell Biol*, 2016) and *Genes & Development* (Starnes *et al*., *Genes Dev*, in press) (in collaboration with the groups of N Mailand and J Daniels). A particular achievement of the Macromolecular Crystallography group (G Montoya) was the resolution of the seven stages of the phosphodiester hydrolysis of a homing endonuclease cleaving its target DNA. This dynamic crystallography approach shows, for the first time, that the two and three ion cleavage mechanism can be combined in a single enzyme.

The highlight of the Protein Function and Interactions group, was establishing an expression system for the production of active human insulin-like growth factor binding proteins (IGFBPs). Being able to study these proteins may help identify targets for the development of novel therapeutics.

Technological advances

The program is also responsible for the Protein Production and Characterization Platform, which supports CPR scientists with research projects requiring protein production and structural–functional protein analysis.

The Platform actively collaborates with the rest of the CPR programs, providing high-quality proteins for many projects. Furthermore, new isolation tags and co-expression approaches have been implemented in the facility and the biophysics team has supported CPR groups in the elucidation of mechanisms using biophysical techniques.

Collaborations and international standing

I co-organized a European Molecular Biology Organization – Universidad Internacional de Andalucía workshop on 'Cell Division: Molecular Machinery and Cancer Targeted Therapies,' in which academics, clinicians, and pharmaceutical company representatives agreed the need for combined hybrid approaches to research novel therapies for cancer. In addition, I was invited to become a panel member for the evaluation of applications for iNextEU project grants, which focus on research access to European structural biology facilities.

"Our work focuses on determining the structural and dynamic interactions of biomolecules and their complexes that are involved in key biological processes."

MACROMOLECULAR CRYSTALLOGRAPHY

MONTOYA GROUP

Professor
Guillermo Montoya (Group Leader)

Associate Professors
Gulnahar Mortuza
Pablo Mesa
Stefano Stella

Postdoc
Bhargav Saligram Prabhakar

PhD Students
Dario Hermida Aponte
Ganesha Pandian Pitchai
Pablo Alcón Hernández
Diana Kowalick
Dan Yang Wang

Technicians and Assistants
Elisabeth Bragado Nilsson (Academic Research Technician)
Saranya Nallapareddy (Research Assistant)
Kamila Kamuda (Technician)
Nika Jachowicz (Laboratory Assistant)

INTERNAL COLLABORATORS

Jeremy A Daniel (Daniel Group, Protein Signaling Program) new DNA damage response factors.

Niels Mailand (Mailand Group, Protein Signaling Program) biophysical characterization of proteins and protein domains.

Jakob Nilsson (Nilsson Group, Protein Signaling Program) biophysical characterization of phosphatase interactors.

Jesper V Olsen (Olsen Group, Proteomics Program) kinase assays and kinase–substrate relationships.

Jiri Lukas (Lukas Group, Protein Signaling Program) fluorescence tagging of endogenous genome caretakers using gene-editing technology.

EXTERNAL COLLABORATORS

Carol Robinson (University of Oxford, UK) mass spectrometry of protein complexes.

Imre Berger (EMBL, Grenoble, France) production of protein complexes.

Christiane Schafitzel (EMBL, Grenoble, France) electron microscopy of protein complexes.

Francisco Blanco [Center for Cooperative Research in Biosciences (cicBiogune), Spain] discovery and characterization of new factors in cellular responses to replication stress.

José María Valpuesta [Spanish National Center for Biotechnology – National center for Biotechnology (CNB-CSIC), Spain] electron microscopy studies of chaperonins.

Marcos Malumbres [Spanish National Cancer Research Centre (CNIO), Spain] structure–function studies of mitotic kinases.

Travis Stracker [Institute for Research in Biomedicine, Barcelona (IRBB), Spain] structure–function studies of kinases involved in the DNA damage response.

Ian Hickson [Center for Chromosome Stability, University of Copenhagen (UCPH), Denmark] the role of recombination-mediated DNA repair in genome integrity maintenance.

Thue Schwartz [Novo Nordisk Foundation (NNF) Center for Basic Metabolic Research, UCPH, Denmark] the structural characterization of novel receptor targets involved in the prevention and treatment of diabetes and obesity. This collaboration has received over 9 million DKK (1.3 million EUR) from the prestigious NNF Challenge Programme.

2015

Challenges and research aims

The Macromolecular Crystallography group studies the structure of macromolecules involved in the cell cycle and genome stability and their interactions. Using this approach, we can explore basic mechanistic questions regarding protein function and the evolutionary relationships between cellular components, and we may discover new and better targets for developing novel therapeutics against diseases such as cancer. However, the current lack of knowledge of macromolecules at the atomic level hampers our full understanding of these biological processes, thereby hindering translational advances. We apply our well-established expertise to combine biophysical and biochemical assays with structural approaches, such as X-ray crystallography and electron microscopy, to pursue our research goals.

Achievements

Having established a state-of-the-art crystallization facility at CPR, in 2015 we introduced new protocols and isolation tags, to improve the purification of protein complexes and large proteins, which will allow us to address complicated scenarios from a structural point of view. In 2015, we also completed the integration of our projects with the production of protein complexes in the Protein Production and Characterization Platform for structural analysis.

The group published eight research articles in 2015. These articles described our research in spindle assembly and our research in DNA-binding proteins where we solved three different crystal structures. One particular highlight was our paper published in *Nature Structural & Molecular Biology* (Molina *et al.*, *Nat Struct Mol Biol*, 2015) in which Stefano Stella and a former postdoc, Rafael Molina, implemented a method for producing biological crystals that allowed us to observe, for the first time, the generation of a DNA double-strand break by the homing endonuclease I-DmoI (see Figure 19). We were able to capture intermediates of the different catalytic steps, and this allowed us to watch the reaction by 'freezing' multiple states. We observed the successive entry of two metals involved in the reaction and the arrival of a third cation in a central position of the active site. This third metal ion has a crucial role, triggering the consecutive hydrolysis of the targeted phosphodiester bonds in the DNA strands and leaving its position once the double-strand break is generated. This information will help to design new enzymes that may be useful in genome editing. This paper is one of the most downloaded that the journal has ever published and is in the top 5% of all research output, as scored by Altmetric. A video describing our findings is available[3]. These findings have been complemented with three follow-up manuscripts, including one using a molecular dynamics approach to fully understand the ion movement in the active center.

In a second key project this year, in collaboration with the group of Francisco Blanco at CICBiogune (Spain), we published a paper in *Nature Communications* (De Biasio *et al.*, *Nat Commun*, 2015) describing how we uncovered the molecular interaction between proliferating cell nuclear antigen (PCNA), the DNA sliding clamp, and the p15 protein, involved in the regulation of DNA replication and repair. We solved the crystallographic structure of this complex, which has enabled us to propose a new mechanism of action of PCNA that facilitates the switch from replicative to translesion synthesis polymerase binding.

STRATEGIC GOALS

- To understand CCT/TRiC control of cell cycle progression
- To elucidate the role of protein interactions in a chromatin context
- To investigate the regulation of the kinase loop in cell division
- To design specific protein–DNA interactions for genome editing
- To decipher the mechanism of DNA unwinding during genome replication

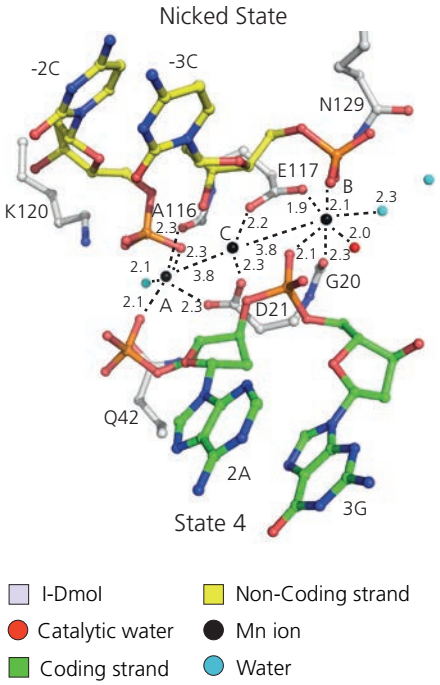


Figure 19 | Detailed view of the active site showing the nicked intermediate of the hydrolytic reaction showing the breakage of the noncoding-strand phosphodiester bond. All distances are shown with dashed lines in angstroms.

3. https://www.youtube.com/watch?v=YFutF3Cqk3U

"We apply our world-leading expertise in protein biochemistry and structural biology to further our understanding of the molecular details in protein–protein interactions."

PROTEIN FUNCTION AND INTERACTIONS

WIKSTRÖM GROUP

Associate Professor
Mats Wikström (Group Leader)

Postdoc
Jesper Langholm-Jensen

PhD Student
Anne Sofie Molsted Wanscher

Master's Student
Sofia Møller

INTERNAL COLLABORATORS

Niels Mailand (Mailand group, Protein Signaling Program) the structure and interactions of novel SUMO-binding motifs.

EXTERNAL COLLABORATORS

Lars Björck (Lund University, Sweden) mechanistic studies of virulence factors from group A streptococci (GAS).

Johan Malmström (Lund University, Sweden) proteomic mass spectrometric analyses of secreted proteins from GAS.

Sten Ohlson (Nanyang Technological University, Singapore) novel therapeutic approaches for the treatment of bacterial infections.

Michael Gerstenberg (Novo Nordisk A/S, Denmark) the interaction matrix for insulin-like growth factor binding proteins.

2015

Challenges and research aims

Structural and functional knowledge of human proteins with therapeutic relevance is needed to design and process the next generation of protein therapeutics. In order to conduct structural and functional investigations, large quantities of recombinant proteins are needed. However, finding a suitable recombinant production system remains a challenge. Our aim in 2015 was to establish such a system for the expression of IGFBPs (insulin-like growth factor blinding proteins), which regulate important cellular processes such as the cell cycle and apoptosis, and may offer therapeutic opportunities in cancer and other diseases.

Bacterial infections represent a major threat to humans, a threat that has become aggravated by the alarming and ongoing increase of antibiotic resistance. Identification of novel preventive, diagnostic, and therapeutic strategies will help to successfully treat bacterial infections in the future. To achieve this goal, we need to better understand the complex molecular interplay between significant bacterial pathogens, such as GAS, and their human hosts, to define the molecular basis for virulence. Worldwide, GAS causes an estimated 700 million cases of mild and non-invasive infections each year, of which approximately 650,000 progress to severe invasive infections with an associated mortality of approximately 25%. In 2014, we determined the complex between the novel GAS virulence determinant sHIP and the antimicrobial protein histidine-rich glycoprotein, and have focused on further understanding this interaction in 2015.

STRATEGIC GOALS

- To develop a eukaryotic expression system for the successful expression of all IGFBPs
- To further characterize the interaction between the novel virulence determinant sHIP from GAS and proteins from the human host

Achievements

We have established a HEK293 expression system suitable for the production of active, full-length human IGFBPs (Wanscher *et al*., *Protein Expr Purif*, 2015), so providing the means for further investigations of the structure and function of the IGFBP interaction matrix, which may help identify targets for developing novel therapeutics (Fig. 20).

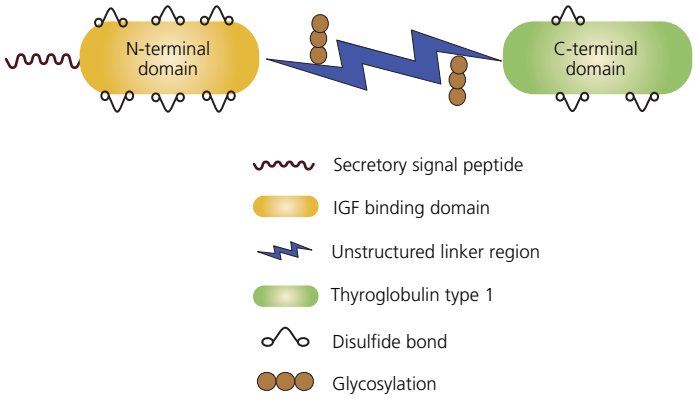


Figure 20 | Overall domain architecture of full-length human IGFBP-1 to -6 including an outline of the location of disulfide bonds and glycosylation sites. The molecular weight (Mw) of full-length human IGFBPs range from 25 to 35 kDa.

PROTEIN PRODUCTION AND CHARACTERIZATION PLATFORM

Professor
Guillermo Montoya (Platform Leader)

Prokaryotic Protein Expression
Andrea Lages Lino Vala (Team Coordinator)
Khalid Pardes (Technician)
Havva Koc (Technician)
Mia Funk Nielsen (Technician)
Mateo Belluci (Technician)
Motiejus Melynis (Technician)

Eukaryotic Protein Expression
Giuseppe Cazzamali (Team Coordinator)
Alison Lilley (Technician)
Tasja Ebersole (Technician)
Michael Ross Williamson (Technician)

Biophysical Protein Characterization
Blanca López-Méndez (Team Coordinator)
Irina Pozdnyakova (Academic Research Technician)
Mille Egeberg Ottosen (Laboratory Assistant)

Proteome-wide analysis of protein–protein interactions, powerful multiple-affinity protein purification methods, and ultrasensitive microscopy have led to the identification of many novel multiprotein complexes involved in all major biological process. Malfunctions of protein pathways that orchestrate cell proliferation and guard the integrity of the genome are emerging as essential in the development of many diseases, including cancer, which are a great burden on individuals and society. Nearly every major process in a cell is carried out by assemblies of 10 or more protein molecules. Moreover, the functions of proteins cannot be understood if we consider them individually and separate from their molecular and cellular contexts.

While this platform has previously developed robust protocols to express and purify single domains and small proteins, in 2015 we expanded these methods to produce proteins and protein complexes that are involved in key cellular processes for structural and mechanistic studies (see Figure 21). This extremely challenging enterprise has resulted in the production of molecular machines with multiple subunits involved in mitosis and the DNA replication complex. We have also produced giant kinases that are involved in mitosis and DNA repair.

In addition, we have been focusing on integrating the Platform even more closely with

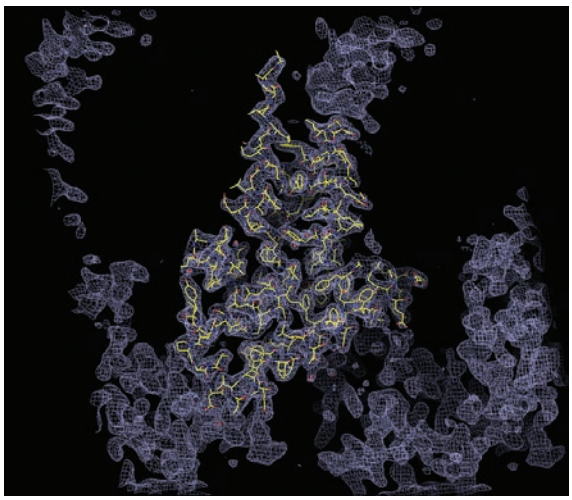


Figure 21 | Electron density map of a crystal structure of a protein complex involved in sister chromatid separation, produced in the facility by Ganesha Pitchal. The crystal structure was solved by Pablo Mesa using multiple anomalous dispersion (MAD).

research projects at CPR, including in-depth biochemical and biophysical characterization for structure–function studies. In addition, we are aiming to include genome editing to tailor some of our hosts for better protein production. During this year, we were extremely pleased to recruit Blanca López-Méndez as Coordinator of the Biophysical Protein Characterization team. Blanca's broad expertise in biophysics and nuclear magnetic resonance spectroscopy complements our technical abilities. Her recruitment will help us to implement new methods and innovative approaches that will allow us to characterize protein complexes and transient protein interactions involved in normal cellular function and disease. Aside from providing an excellent service, the members of the facility have contributed to a number of publications in collaboration with CPR colleagues and international groups.

EDUCATION AND CAREER DEVELOPMENT

One of our key missions is to train, educate, and develop the careers of CPR's junior researchers – more than half of CPR's current employees are junior researchers, including postdocs, PhD students, and master/bachelor students – so that they become exceptional protein scientists. To that end, we aim to create an unmatched career development portfolio to attract the most talented young scientists, enable them to reach top international levels early on in their careers, and provide them with unique skills to compete for leadership positions both in academia and industry.

DEVELOPING THE 'COMPLETE PROTEIN SCIENTIST'

Our vision of a 'complete protein scientist' is best described as a person equipped with skills in a broad range of complementary protein technologies and capable of applying them to address fundamental challenges in academic research, the biotech/pharmaceutical industry, or in the public healthcare sector. Additional hallmarks of a 'complete' scientist include good scientific practice, intellectual understanding, entrepreneurship with ambition for continuous innovation, and the ability to create innovative opportunities and provide novel solutions.

"Teaching is a great way to make the next generation of scientists aware and involved in your discipline and teaching opportunities frequently lead to new scientific collaborations."

LARS J JENSEN

CPR is part of the Copenhagen Bioscience PhD program, which is a new recruitment initiative from the Novo Nordisk Foundation (NNF). The program offers talented students with a university degree from abroad the opportunity do a PhD at one of the NNF research centers. Sixteen students will be recruited annually through the program for enrolment in August the same year, with four students per year at CPR. Further details on the NNF website[4].

In 2015, we continued previously launched career development initiatives such as the Research in Progress (RIP) meetings that take place 3–4 times per month. These meetings allow our scientists to present their unpublished results to their peers at CPR, providing an opportunity for them to develop their presentation skills and invite input from their colleagues.

A top-tier research center is based on a continuous flow of young scientists in short-term positions and it is our aim that talented scientists perceive CPR as an excellent career

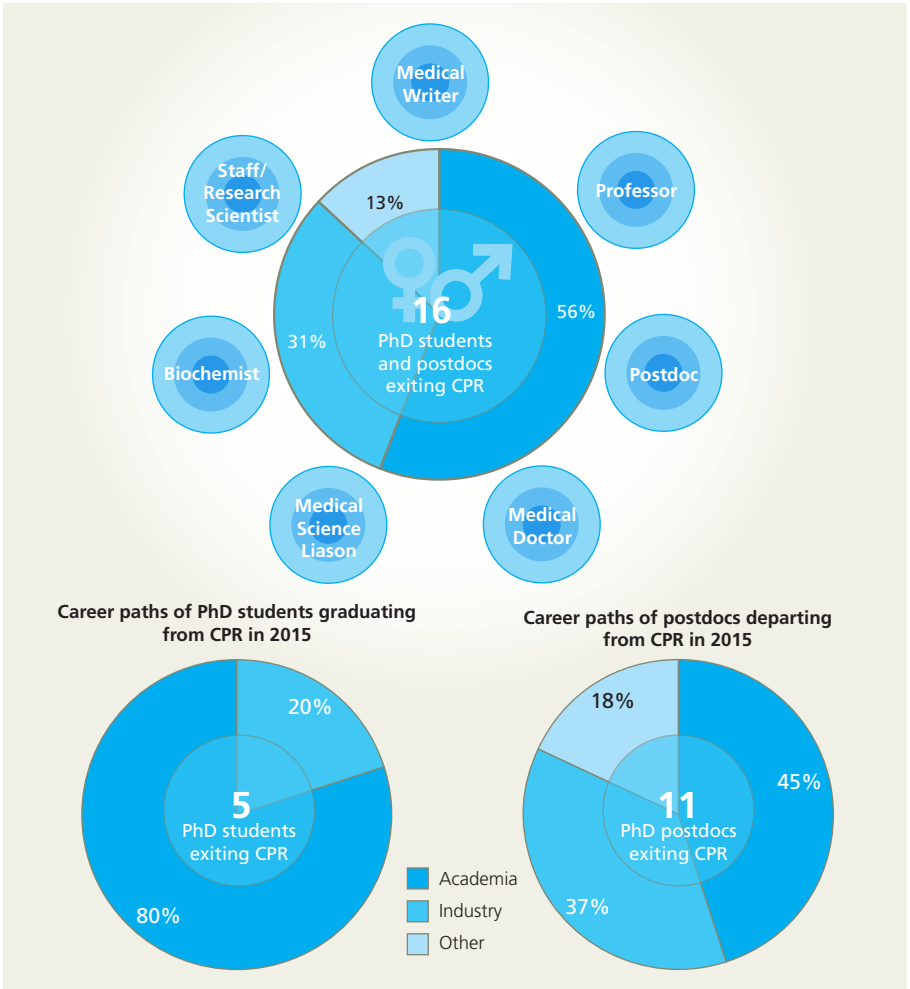


Figure 22 | (top) Positions taken up by PhD students and postdocs leaving CPR in 2015; (bottom left) Career tracks of PhD students and (bottom right) postdocs leaving CPR in 2015.

4. http://www.novonordiskfonden.dk/da/content/copenhagen-bioscience-phd-programme

move. In 2015, five PhD students completed their education here and moved on to the next stage of their career and 11 postdocs left CPR (Figure 22). We are pleased that the number of CPR researchers securing influential positions outside CPR is increasing. Our policy to create complete protein scientists who are sought after by academia and industry also secures rotation of scientific staff, avoids scientific 'inbreeding,' and develops CPR's image as a hub for both attracting new talents and also developing them into well qualified protein experts who are highly sought after. We have also planned an alumni meeting for 2016, with the goal of providing a forum for CPR alumni to exchange experiences, build bridges between academic and industrial protein research, identify new areas for collaboration, and cultivate a 'common language.'

EDUCATIONAL ACTIVITIES

Student supervision, PhD and master/ bachelor teaching activities (Table 1) are vital components of the CPR strategy to identify and develop talented students into complete protein scientists. Education also provides career development opportunities for CPR faculty and scientists in areas such as teaching, curricular development, e-learning, and related activities. In addition, teaching and student supervision is an important part of facilitating one of the goals set by the Dean, which is a seamless integration of CPR into the educational and research activities of the Faculty of Health and Medical Sciences and the wider University.

The Education Coordinator Professor Amilcar Flores-Morales coordinates teaching at CPR. He is responsible for all aspects of the implementation of CPR-initiated PhD courses at the Faculty, with tasks that range from curricular development to coordination with educational program leaders at the University of Copenhagen (UCPH). CPR's

AMILCAR FLORES-MORALES, EDUCATION COORDINATOR

main themes: student supervision, organization and implementation of master's and PhD courses within UCPH, and teaching and student evaluation by the CPR faculty on national and international courses. In 2015, 46 PhD students and 10 Master's students were supervised by the CPR faculty.

CPR offers two Master/bachelor courses within the MSc Human Biology program: 'Bioinformatics and Systems Biology for Human Biologists' (organized by Søren Brunak with input from members of the Disease System Biology Program), and 'Advanced Methods for the Analysis of Protein Disease Mechanisms' (organized by Amilcar Flores-Morales, with input from most of CPR's Group and Platform Leaders. For its next edition, this course will be a part of the UCPH summer school program, with the aim of recruiting ambitious students both nationally and internationally. The curriculum is also being modified to include an e-learning module to be developed in collaboration with UCPH's Centre for Online and Blended Learning. In 2015, Lars J Jensen initiated a new CPR-led course, 'Introduction to Bioinformatics', which is a part of a new educational program 'Quantitative Biology and Disease Modeling,' (co-organized by the Faculty and the Technical University of Denmark). This year we also offered the third edition of the PhD course 'Ubiquitin and Ubiquitin-like modifiers.' This biannual course is organized by Neils Mailand, Jakob Nilsson, and Simon Bekker-Jensen, with contributions from several CPR faculty members,

as well as with highly regarded external speakers from Denmark and abroad.

In addition to CPR-initiated educational activities, CPR's faculty has also contributed to several programs within the Faculty, as well as internationally. For example, Lars J Jensen participated in European Molecular Biology Organization (EMBO) practical courses in computational biology in Japan and Israel. Jesper V Olsen, Søren Brunak, and Lars J Jensen were speakers at the Copenhagen Summer School: 'Personalized Medicine – the future treatment paradigm.' In addition, Michael Lund Nielsen and Jakob Nilsson contributed to educational programs in the Faculty of Science in the areas of protein mass spectrometry and mitosis and cell division, respectively.

Table 1 | Overview of teaching activities 2015

	Type of course	Topic
Nilsson group	Pre-graduate course, Master's level	Human Genetics (Department of Biology, University of Copenhagen, Denmark)
	Pre-graduate course, Master's level	Cell Cycle Regulation and Cancer (Department of Biology, University of Copenhagen, Denmark)
Olsen group	Pre-graduate course, Master's level	Personalized Medicine – the future treatment paradigm (Copenhagen Summer University for working professionals. 17–21 August, Frederiksberg Campus, Copenhagen, Denmark)
	PhD course	9th Proteomics Summer School (Brixen, Italy)
Jensen group	Pre-graduate course, Master's level	Introduction to Bioinformatics (Copenhagen, Denmark)
	Pre-graduate course, Master's level	Honors Student Seminar (Osaka, Japan)
	Pre-graduate course, Master's level	Bioinformatics for Human Biologists (Copenhagen, Denmark)
	Pre-graduate course, Master's level	Chemoinformatics in Drug Discovery (Technical University of Denmark)
	PhD course	Bioinformatics Spring School (Bertinoro, Italy)
	PhD course	EMBO Practical Course on Computational Biology (Okinawa, Japan)
	PhD course	Molecular Mechanisms of Disease Summer School (Copenhagen, Denmark)
	PhD course	In silico Drug Discovery (Warsaw, Poland)
	PhD course	EMBO Practical Course on Computational Analysis of Protein–Protein Interactions (Norwich, UK)
	PhD course	The Literature Text Mining Approach in Cancer Research (Safed, Israel)
Brunak group	Pre-graduate course, Master's level	Bioinformatics and Systems Biology for Human Biologists
	Pre-graduate course, Master's level	Introduktionskursus i IT&Sundhed
	Copenhagen Summer School	Personalized Medicine – the future treatment paradigm (Copenhagen Summer University for working professionals. 17–21 August, Frederiksberg Campus, Copenhagen, Denmark)
Nielsen group	Pre-graduate course, Master's level	Advanced Protein Science I (Department of Biology, University of Copenhagen, Denmark)
	Pre-PhD course, Master's level	Mass Spectrometry Coupled to Separation Techniques in Bioanalytical Chemistry (Drug Research Academy, University of Copenhagen, Denmark)
Amilcar Flores-Morales	Pre-PhD course, Master's level	A survey of Molecular Endocrinology (Karolinska Institute, Stockholm)

SEMINARS AND ANNUAL RETREAT

CPR seminar series

The purpose of our seminar series (Table 2) is to attract the most distinguished researchers from around the world to talk about their work, and give our junior scientists the opportunity to learn from first-class scientists. The seminars are attended by the majority of students and scientists at CPR, and sessions with individual researchers can be arranged if requested.

SPA annual retreat

In August 2015, the first annual retreat of CPR's Student and Postdoc Association was held at Hotel Marienlyst in Helsingør and 25 enthusiastic PhD students and postdocs participated in the event. The goal of the retreat was to tighten scientific and social bonds between young CPR scientists, who were able to present and discuss their projects in a relaxed environment. All participants agreed that it was a great opportunity to get to know their colleagues and discuss the different research questions they are working on. Moreover, many people were inspired to strive to establish collaborations between the different departments within CPR. We consider the first SPA retreat a great success and are looking forward to the next opportunity to foster scientific relationships within CPR.

Table 2 | Seminars arranged by CPR in 2015

Date	Title	Speaker	Affiliation	Hosted by
Jan 13	Nuclear organization in DNA repair	Evi Soutoglou	Institut de Genetique de Biologie Moleculaire dt Celluraire (IGBMC), France	Claudia Lukas
Feb 2	The physiology of nutrient sensing by mTOR	Alejo Efeyan	Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, USA	Jiri Lukas
Feb 6	Structural insight into RNA polymerase I and III transcription	Christoph Müller	European Molecular Biology Laboratory, Germany	Guillermo Montoya
Feb 24	How to make a cilium by intraflagellar transport	Esben Lorentzen	Max Planck Institute of Biochemistry, Germany	Jakob Nilsson
Mar 26	Deep imaging to explore genome architecture and function	Tom Misteli	Center for Cancer Research, National Cancer Institute, USA	SPA
May 26	Guarding the genome during V(D)J recombination: functional redundancies between RAG, DNA repair, and DNA damage response machineries	Ludovic Deriano	Pasteur Institute, France	Jeremy Daniel
Jun 25	Varying the terrain of epigenetic landscapes: implications for human cancer	David Allis	Rockefeller University, USA	SPA
Sep 1	A SAGA of Gcn5 and USP22	Sharon YR Dent	Center for Cancer Epigenetics, The University of Texas MD Anderson Cancer Center, USA	Jeremy Daniel
Sep 22	Mechanism of DNA–protein crosslink repair in S-phase	Julien Duxin	Howard Hughes Medical Institute, Harvard Medical School, USA	Jiri Lukas
Oct 20	Regulation of the protein phosphatase scaffold RepoMan in mitosis	Mathieu Bollen	University of Leuven, Belgium	Jakob Nilsson
Nov 3	Nuclear ADP-ribosylation mediates rapid chromatin remodeling	Andreas Ladurner	Biomedical Center Munich, Germany	Claudia Lukas
Nov 25	How cohesin controls sister chromatid cohesion and chromatin structure	Jan-Michael Peters	Research Institute of Molecular Pathology, Vienna, Austria	Simon Bekker-Jensen

INTERVIEW WITH ALEXANDER HOGREBE, PHD STUDENT, OLSEN GROUP (PROTEOMICS PROGRAM)

Why CPR?

When I came to CPR for an interview, I was amazed by the broad spectrum of expertise focused in a single center. CPR offers unique opportunities for a PhD student of life sciences by uniting cutting-edge research technology, core facilities, and experts from very different fields on just three floors. That, combined with a very welcoming atmosphere in my new host lab, reassured me that these were excellent conditions to start a thesis, and I haven't been disappointed so far.

What makes CPR attractive?

The great thing about CPR for a young researcher like me is that people with completely different backgrounds can get together for collaborations and the exchange of scientific knowledge. This happens at all levels, not only when we present our research to our fellow students and postdocs but also on a daily basis. People here are very happy to share their knowledge, and you easily get caught up in the positive atmosphere of scientific exploration. And after work on a Friday, afternoon discussions continue over a beer in our science square.

How did you hear about CPR?

CPR, and its Proteomics Program in particular, was recommended to me by my former Master's thesis supervisor Professor Bernard Küster in Germany, and this recommendation underlines CPR's good international reputation.

Has your impression of CPR as a research institution changed now that you are here?

My initial impression of a welcoming atmosphere has been confirmed, especially when I joined SPA, CPR's student and postdoc association. The members of SPA are actively searching for intensive scientific exchange which, in my opinion, is really important in an academic environment.

What do you expect your PhD studies here to give you?

I expect (and already experience) an excellent scientific environment that enables me to pursue the questions emerging as part of my research thesis. I think that this will help me to turn into a well-prepared researcher. At the end of my 3 years in the PhD program, I am sure that I will have established many connections to, and collaborations with, researchers from other fields, both within and outside CPR.

OUTREACH AND DISSEMINATION

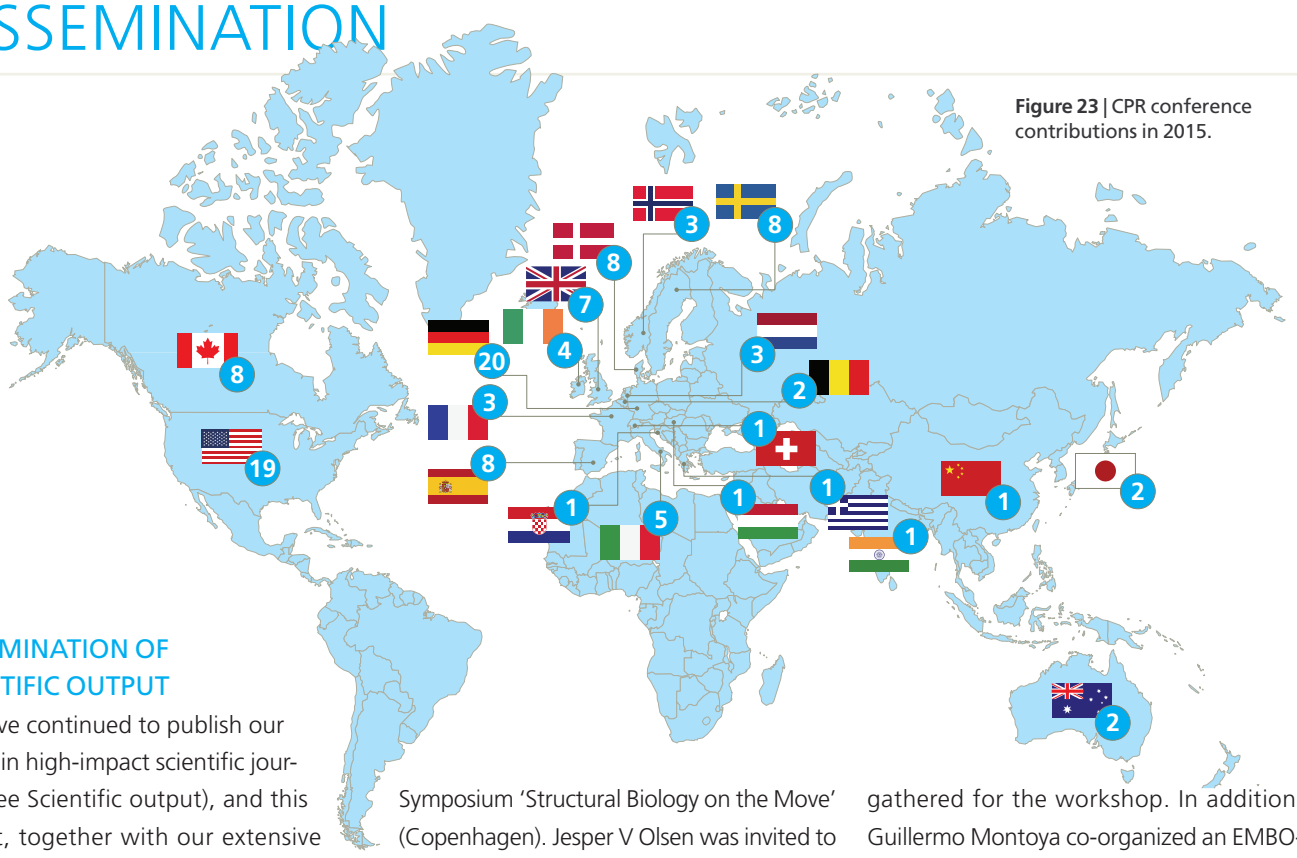


Figure 23 | CPR conference contributions in 2015.

DISSEMINATION OF SCIENTIFIC OUTPUT

We have continued to publish our results in high-impact scientific journals (see Scientific output), and this output, together with our extensive network of collaborators and alumni, has resulted in the high global visibility and standing in protein research that CPR has today. The world-leading position of CPR is evidenced by the number of conferences our scientists organize and are invited to speak at, and these activities also ensure wide dissemination of our scientific output.

Scientists from CPR have been invited to speak at many high profile conferences (Table 3), which increases the visibility of CPR research and fosters interactions internationally (Figure 23). For instance, Jiri Lukas was invited to talk at diverse conferences in 2015, including the prestigious Curie lecture (Paris, France) and the Ernst Caspari lecture (Gottingen, Germany). Guillermo Montoya was a keynote speaker at several conferences in 2015, including the European Molecular Biology Organization – Universidad Internacional de Andalucía (EMBO-UNIA) workshop in 'Cell Division: Molecular Machineries and Cancer Targeted Therapies' and the Benzon Symposium 'Structural Biology on the Move' (Copenhagen). Jesper V Olsen was invited to speak at nine conferences in 2015, including a Keystone Symposium on 'The Human Proteome' (Stockholm, Sweden) and also at the 14th Human Proteomics Organization World Congress (Vancouver, Canada), where he chaired a session on 'Phosphoproteomics and Cell Signaling.' Finally, from the Disease Systems Biology Program, Søren Brunak was invited to give 12 talks at conferences including at the Annual Danish Bioinformatics Conference and the 10th Annual Meeting of the International Conference on Genomics (China).

Our research leaders also organized several conferences in 2015. These included the Keystone Symposium 'Genomic Instability and DNA Repair' (Jiri Lukas) and the EMBO Workshop 'Dynamic Kinetochore' (Jakob Nilsson). The 'Dynamic Kinetochore' workshop was sponsored by competitive grants from EMBO and the Carlsberg Foundation. In total, 90 people from around the world working on different aspects of cell division gathered for the workshop. In addition, Guillermo Montoya co-organized an EMBO-UNIA workshop on 'Cell Division Molecular Machinery and Cancer Targeted Therapies,' in which academics, clinicians, and pharmaceutical company representatives agreed on the need for combined hybrid approaches to research novel therapies for cancer. Furthermore, Søren Brunak organized and chaired a session entitled 'Cancer Panomics: Integrative analysis of cancer high-throughput 'omics' data to enable precision oncology' at the Pacific Symposium on Biocomputing (USA), and Niels Mailand was Chairman of an EMBO Conference on 'DNA Damage Responses in Cell Physiology and Disease' (Cape Sounio, Greece).

Table 3 | Conference contributions

Invited/keynote speaker	57
Regular talk	16
Poster	38
Organizer	3
Chair	5
Other	7

ESTABLISHING CPR'S IDENTITY

In 2015, we have continued our efforts to establish a stronger identity for CPR, both internally and externally: reaching out to our staff and fellow researchers within our specializations, as well as to our broader academic, industry, and clinical partners, and the general public. In 2015, we have hosted a number of visits from international foundations and organizations, such as management and board representatives from both the Health Axis Europe and the Wellcome Trust. Our visitors left CPR with a strong impression of how our facilities and our highly competent staff together create a leading research center for protein research at the University of Copenhagen (UCPH).

Collaborations

The details of the many important international collaborative projects taking place at CPR are given in the Program sections of this Annual Report. Such activities have boosted our exposure, given us new and extended possibilities for branding CPR and our research to the outside world, and have also provided a strong base on which to build further scientific relationships and collaborations.

In 2015, we consolidated our status as an unmatched partner in protein science by strengthening the infrastructure of CPR. The specialized expertise of CPR's established technology Platforms, interlinked directly with the research groups, has ensured knowledge and collaboration synergy across the whole of CPR, as evidenced by our exceptional scientific output. Internally, the Program/Platform structure fosters collaborative research projects at CPR and creates a strong sense of cohesion and belonging. Externally, the Platforms are central to the CPR brand and promote CPR as an important hub of protein technological excellence among our peers, collaborators, potential recruits, and alumni.

CPR fosters collaborative efforts across the Novo Nordisk Foundation (NNF) Center Cluster, NNF Laureates, and other centers at the Faculty of Health and Medical Sciences based on mutual scientific interests. Prominent ongoing examples include the advanced Protein Imaging Platform and flow cytometry expertise, both shared with DanStem, a joint chemoinformatics position (Thomas Frimurer) between CPR (Søren Brunak) and the Basic Metabolic Research Center (Thue Schwartz), and several common projects with the Center for Healthy Aging (Ian Hickson). Equally important, we are also initiating collaborations with industry – an example of this is the sharing of an Industrial PhD between the Olsen group and Novo Nordisk A/S. Scientists from the Choudhary group are working with NNF Laureate Stephen Cohen, also from UCPH, on investigating the function of ubiquitylation in HIPPO signaling, and we are also initiating collaboration between Matthias Mann and the Steno Diabetes Center. The recently established collaborations with Danish and Scandinavian hospitals, with Søren Brunak using clinical data, such as electronic patient records and medication histories, and collection of samples from large subpopulations, also add to these budding efforts.

Internal communication

The identity of CPR is also firmly anchored in the world-class research environment that we provide for our scientists. In particular, our young scientists are given the opportunity to learn from our expert senior scientists. Our popular CPR-wide seminars, Research in Progress meetings and CPR Center Meetings,

"With our enhanced communication initiative, we intend to boost the exposure of CPR as an international hub for specialized protein research."

LOTTE SKIPPER,
COMMUNICATIONS MANAGER

in which news about CPR and its staff is combined with presentations of strategies and innovative strategic initiatives, build and support a feeling of identity and belonging among our staff.

Actions have been taken in 2015 to strengthen internal communication, with the goal of improving both the flow and quality of information coming from CPR management, the Faculty of Health and Medical Sciences, and the University. We recognize that it is vital to adapt and tailor information from these three sources to meet the information needs of our international employees, especially those who are new to the Danish system and culture. By monitoring and adapting Faculty and University news, we can disseminate the most relevant and important information to our staff via a number of internal communication channels:

- CPR intranet portal
- CPR information boards located on each floor displaying practical information about CPR/Faculty/University issues, CPR events, and news about CPR's employees
- Verbal, written, and electronic communication from CPR group leaders to their own group members
- CPR's annual report to emphasize the vision and mission of CPR, highlight

CPR's achievements, and affirm our strong focus on the people who make up CPR, further fostering a sense of belonging

Strengthening the CPR brand

During the next six months we will be working with the science communications consultancy Elevate Scientific and the communications department at the Faculty to strengthen the CPR brand even further. The project involves a thorough audit of our website to launch renewed and improved web pages with a strong focus on adaptation to our audiences, user-friendliness, visually appealing graphical layout, and on branding CPR. At the same time, we will expand CPR's presence on our social media channels (CPR alumni Facebook page, Twitter, and LinkedIn) and initiate a more dialog-oriented approach to our communications.

MEDIA COVERAGE AND PUBLIC ENGAGEMENT

We continuously strive to raise the profile of the CPR brand externally by generating high-quality scientific results and disseminating these through CPR's website, the Faculty's global website, via social media, and in printed publications. Our goal is to convey the value of CPR to diverse audiences in a clear and professional manner. At the same time, we are progressing towards developing CPR as a hub not only for protein science but also for communication about protein science.

In 2015, we wrote and distributed 19 press releases and other news stories, covering breakthrough research, grants, and awards won by CPR staff and other major developments, such as the establishment of Matthias Mann's new research group in Clinical Proteomics. Our most exciting news stories were

Figure 24 | (left) News reporting of Niels Mailand's group publishing in *Nature*, Jyllands-Posten (8 May 2015); (middle) news reporting of Matthias Mann's NNF grant, *Politiken* (16 Sep 2015); (right) profile feature on Matthias Mann and Søren Brunak's research, *Nature* (5 Nov 2015).

distributed via the Faculty communications department to influential scientific journalists, and signposted using CPR's social media platforms and as news content on our website. Via the Faculty's extensive network of science journalists, we have been able to 'communicate' some of our news stories to specific news media both in Denmark and internationally. Many of our news stories have also been disseminated on Twitter (via CPR's own Twitter feed, as well as the feed of the Faculty) and disseminated to our alumni via our Facebook or LinkedIn pages.

An example of a story that got both Danish and international coverage was the publication of a research paper in *Nature* by Niels Mailand and researchers in his group, describing the discovery that ubiquitin-dependent modification of H1-type linker histones has a central role in how cells repair cytotoxic DNA lesions (Thorslund *et al.*, *Nature*, 2015).

Nationally, the story was featured in the Danish newspaper *Jyllands-Posten* (Figure 24).

It quickly spread in the international science media, and was featured in the *Dispatch Tribunal*, *Tech Times*, *Genetic Engineering & Biotechnology News*, and on *NDTV*.

Jesper V Olsen featured in a Danish radio program (Videnskabens verden, DR1) on the Human Proteome atlas and mass spectrometry. In the interview, Professor Jesper V Olsen was invited to the program to discuss recent milestone papers in *Nature* on the initial draft of the human proteome [*Nature* **509**, p. 575, (2014) and *Nature* 509, p. **582**, (2014)].

The large NNF grant secured by Matthias Mann to set up his new research group at CPR was announced on the CPR website[5]. This story was picked up by the Danish press (*Politiken*, Figure 24).

In November 2015, the Faculty of Health and Medical Sciences had a profile feature in *Nature's* Spotlight on Denmark-supplement, where the Faculty presented both the research conducted by Professor Matthias Mann and Professor Søren Brunak from CPR along with other prominent researchers from the Faculty (*Nature* 5 Nov 2015, Figure 24).

We have also been asked to display our center to architects, TV journalists/producers, and photographers, who have all shown great interest in our research and our excellent facilities. In addition, the facilities of CPR have been used for a trailer for a children's series called 'Borneforskerne' *(The Child Researchers)*, produced by Danish Broadcasting (DR)[6].

5. http://www.cpr.ku.dk/cpr-news/ambitious-new-biomedical-research-program-at-the-novo-nordisk-foundatin-center-for-protein-research/
6. https://www.dr.dk/tv/se/boern/ultra/boerneforskerne/boerneforskerne-1

RESEARCH GRANTS AND AWARDS

FINANCIAL STRATEGY

After a highly positive scientific assessment in 2014, the Novo Nordisk Foundation (NNF) awarded CPR an additional grant of 180 million DKK (24.2 million EUR) in January 2015. CPR has now been awarded a total of 780 million DKK (104 million EUR) from NNF, and the grant period has been extended until the end of 2019.

CPR aims to maintain an annual turnover in the 2015–2019 NNF funding period of at least 110 million DKK (14.7 million EUR), with the NNF contribution making up 50% or more of the total funding for CPR.

OVERVIEW OF FUNDING SECURED

In 2015, CPR was able to maintain its turnover at the same level as in previous years, at about 117 million DKK (15.6 million EUR). Of the turnover in 2015, the NNF's contribution was about 60 million DKK (8 million EUR), as expected. Approximately 50 million DKK (6.7 million EUR) was provided by other external funding, and the residual turnover came from the University of Copenhagen (UCPH) (Figure 25).

CPR's strategies for attracting funding

Table 4 | Funding secured in 2015

Funder	Project	Recipient	Type	million DKK	million EUR
Novo Nordisk Fonden	Grant agreement	Ralf Hemmingsen	Danish Private Grant	180.0	24.0
Novo Nordisk Fonden	Mass spectrometry based discovery of blood protein biomarkers for preventive and predictive medicine: focus on type 2 diabetes, obesity and the metabolic syndrome	Matthias Mann	Danish Private Grant	60.0	8.0
Lundbeck Foundation Fellowship	P38-dependent stress responses in human ageing	Simon Bekker-Jensen	Danish Private Grant	10.0	1.3
Novo Nordisk Fonden	Novel receptor targets in the prevention and treatment of diabetes and obesity	Guillermo Montoya	Danish Private Grant	9.8	1.3
Kræftens Bekæmpelse	Histon H1 i det cellulære forsvar mod genom-instabilitet og kræft	Niels Lind Mailand	Danish Private Grant	3.6	0.5
Lundbeck Foundation	RIMMI - Regional Immune-Modulation of Mucosal Inflammation	Søren Brunak	Danish Private Grant	2.8	0.4
Kræftens Bekæmpelse	Analyse af Mastl, en ny central aktør i kræft	Guillermo Montoya	Danish Private Grant	1.8	0.2
Kræftens Bekæmpelse	Kortlægning af TACC3 proteinets biologi: En ny metode til at modvirke kromosom instabilitet	Jakob Nilsson	Danish Private Grant	1.5	0.2
Novo Nordisk Fonden	Chromatin-mediated regulation of DNA double-strand break repair	Niels Lind Mailand	Danish Private Grant	1.5	0.2
Lundbeck Foundation	Elucidating mechanisms for XRCC4-like factor function during DNA double-strand break repair	Valentyn Oksenych	Danish Private Grant	1.4	0.2
Kræftens Bekæmpelse	Proteomics-baseret identifikation af funktionelle proteiner ved DNA-skader i primære lymfocytter	Jeremy Austin Daniel	Danish Private Grant	1.0	0.1
Innovationsfonden	Big Temp Health	Søren Brunak	Danish Public Grant	5.1	0.7
Styrelsen for Forskning og Innovation (FI)	Elucidating the role of replicative stress in hematopoietic stem cells	Ewa Katarina Ohlsson	Danish Public Grant	2.9	0.4
Det Frie Forskningsråd Sundhed og Sygdom (FSS)	An integrated high-throughput approach to uncover the molecular resistance to PARP inhibitors in cancer cells	Michael Lund Nielsen	Danish Public Grant	2.6	0.3
Det Frie Forskningsråd Sundhed og Sygdom (FSS)	Defining mechanisms of mitotic exit and blocking these to target cancer cells	Jakob Nilsson	Danish Public Grant	2.6	0.3

table continued on next page

were successful in 2015 (Table 4). In 2013, CPR attracted external funding amounting to approximately 30 million DKK (4 million EUR), in 2014 the value was 70 million DKK (9.3 million EUR), and in 2015 the value was approximately 150 million DKK (20 million EUR). Of particular note, 60 million DKK (8 million EUR) was granted by the NNF to Program Director Matthias Mann, to set up the Clinical Proteomics group in the Proteomics Program and conduct mass spectrometry-based discovery of blood protein biomarkers for preventive and predictive medicine, with a focus on type 2 diabetes, obesity, and the metabolic syndrome. The turnover in 2016 and, most likely also in subsequent years, is expected to be at least 130 million DKK (17 million EUR).

Our scientists have succeeded in securing funding from many sources in 2015 (Table 4). One highlight was Søren Brunak's group (Disease Systems Biology Program) securing 5.7 million DKK (0.8 million EUR) in funding for the Horizon 2020 project, EU-ToxRisk, which will focus on the interaction between chemicals and protein targets. This project has more than 35 partners around Europe and includes

Table 4 (continued)

Funder	Project	Recipient	Type	million DKK	million EUR
Det Frie Forskningsråd Natur og Univers (FNU)	How the kinetochore generates a "wait anaphase" signal	Jakob Nilsson	Danish Public Grant	1.9	0.3
Lundbeck Foundation PHD Scholarship	Novel roles for PBK mediated phospho-signaling in breast cancer cells	Alexander Hogrebe	Danish Public Grant	1.6	0.2
Styrelsen for Forskning og Innovation (FI)	EliteForsk-prisen 2015	Niels Lind Mailand	Danish Public Grant	1.0	0.1
Styrelsen for Forskning og Innovation (FI)	Sapere Aude bonus / Elucidating the role of replicative stress in hematopoietic stem cells	Ewa Katarina Ohlsson	Danish Public Grant	0.5	0.1
Innovationsfonden	Electrochemical-reduction for improved top-down mass spectrometry - PhD-stipend	Jesper Velgaard Olsen	Danish Public Grant	0.4	0.05
ERC Consolidator Grant	DUB-DECODE	Chunaram Choudhary	EU Grant	14.7	2.0
ERC Starting Grant	Dissecting the constraints that define the eukaryotic DNA replication program	Luis Toledo	EU Grant	11.3	1.5
The EU Framework Programme for Research and Innovation	Mass spectrometric technology for next generation proteomics in systems medicine	Matthias Mann	EU Grant	10.5	1.4
EU Innovative Medicines Initiative 2 Programme	INNODIA	Søren Brunak	EU Grant	6.2	0.8
ESPON - European Commission	EU-ToxRisk	Søren Brunak	EU Grant	5.7	0.8
EU Innovative Medicines Initiative 2 Programme	Rhapsody	Søren Brunak	EU Grant	3.6	0.5
EU Health Programme	EU MedBioinformatics	Søren Brunak	EU Grant	2.3	0.3
Wellcome Trust	Exploring how endocytic recycling of receptor tyrosine kinases specifies cellular responses	Chiara Francaville	International Grant	12.3	1.6
The Regents of the University of New Mexico	Illuminating the Druggable Genome	Søren Brunak	International Grant	1.8	0.2
Her Majesty the Queen of Denmark	Order of Dannebrog Knights Cross	Matthias Mann	Prestigious award		

close collaboration between the Brunak group, Université Paris Diderot, and the University of New Mexico. The Montoya Group is collaborating with the group of Thue Swartz from UCPH on the structure–function characterization of novel receptor targets involved in the prevention and treatment of diabetes and obesity, and has received 9.8 million DKK (1.3 million EUR) from the prestigious NNF Challenge Programme for this purpose. In addition, Chunaram Choudhary (Proteomics Program) received a European Research Council (ERC) Consolidator Grant to investigate deubiquitylase-regulated signaling in human cells, and Luis Toledo (Protein Signaling Program) received an ERC Starting Grant of 11.3 million DKK (1.5 million EUR).

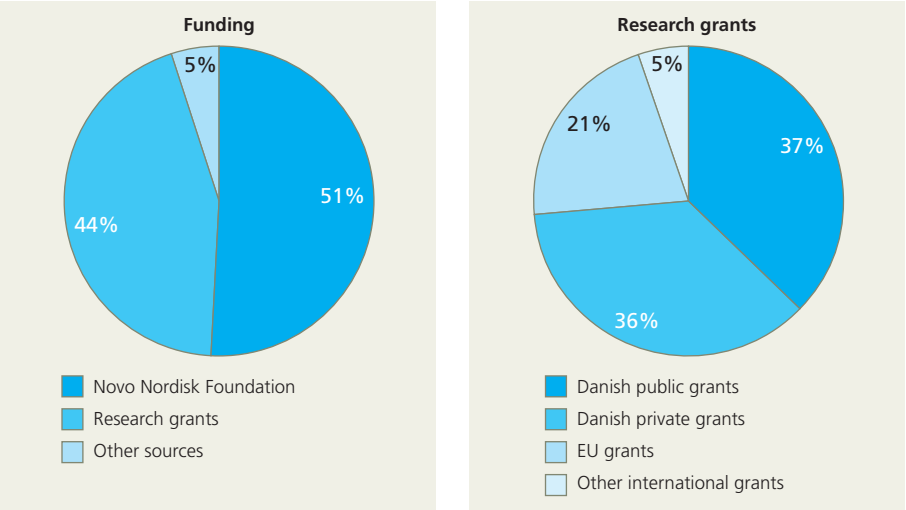


Figure 25 | (left) Breakdown of Novo Nordisk Foundation vs. other funding; (right) breakdown of the type of research grants awarded.

SCIENTIFIC OUTPUT: BIBLIOMETRICS

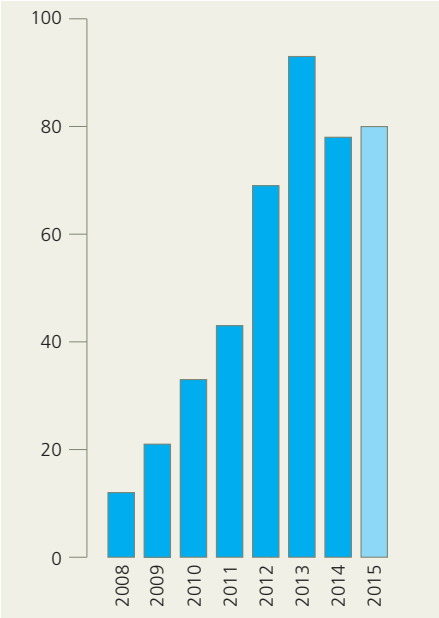


Figure 26 | In 2015, 80 papers were published by CPR scientists.

Table 5 | H-index of CPR's Group Leaders (GL), Platform Leaders (PL), Program Directors (PD), as well as the Executive (ED) and Vice (VD) Directors (data derived from Scopus).

Position	Name	Cited publications	Citations	h-index
ED, PD, GL	Jiri Lukas	197	26,703	87
VD, GL	Jesper V Olsen	126	15,954	52
PD, GL	Søren Brunak	243	38,249	71
GL	Chunaram Choudhary	52	4,433	31
GL	Jeremy Daniel	24	1,844	19
GL, PL	Lars J Jensen	138	14,554	51
PL	Claudia Lukas	47	8,316	32
GL, PL	Niels Mailand	62	4,968	34
PD, GL	Matthias Mann	564	113,079	163
PD, GL, PL	Guillermo Montoya	91	2,368	26
GL, PL	Michael L Nielsen	68	4,783	31
GL	Jakob Nilsson	45	1,371	16
GL	Mats Wikström	34	1,111	23

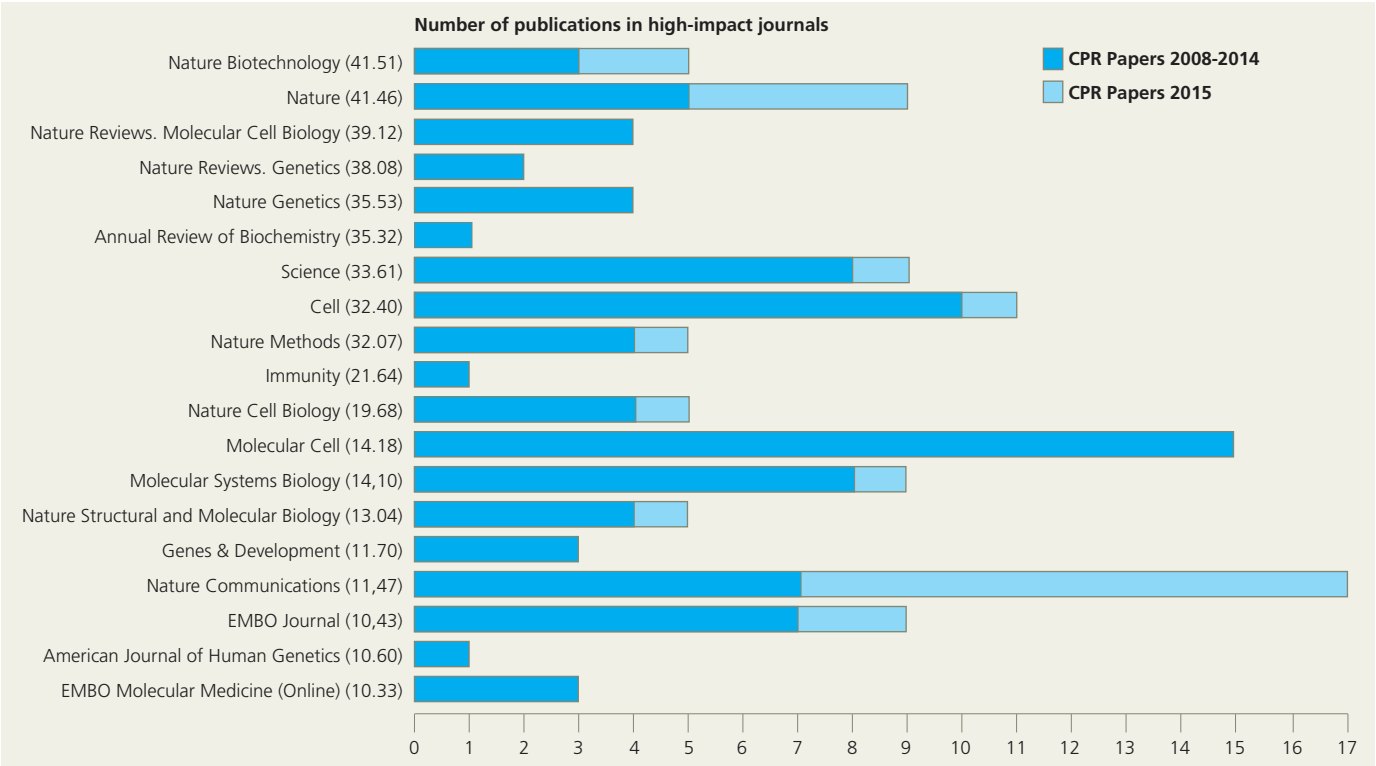


Figure 27 | CPR Papers published in journals with impact factor ≥10 (impact factors given in brackets). In 2015, 24 of CPR's papers were published in journals from prestigious publishing houses such as *Nature*, *Science* and *Cell*[7,8].

7. http://www.bioxbio.com/if/ 8. http://www.nature.com/npg_/company_info/impact_factors.html

SCIENTIFIC OUTPUT: 2015 PUBLICATIONS

CPR authors are highlighted in bold. Publications 2015. Ordered by: first author surname (alphabetically).

- Proteomics
- Disease System Biology
- Protein Signaling
- Protein Structure and Function

Paradoxical resistance of multiple myeloma to proteasome inhibitors by decreased levels of 19S proteasomal subunits

Acosta-Alvear D, Cho MY, Wild T, Buchholz TJ, Lerner AG, Simakova O, Hahn J, Korde N, Landgren O, Maric I, **Choudhary C**, Walter P, Weissman JS, Kampmann M. *eLife*; 4, e08153 (2015)

Genome-wide association study identifies three novel genetic markers associated with elite endurance performance

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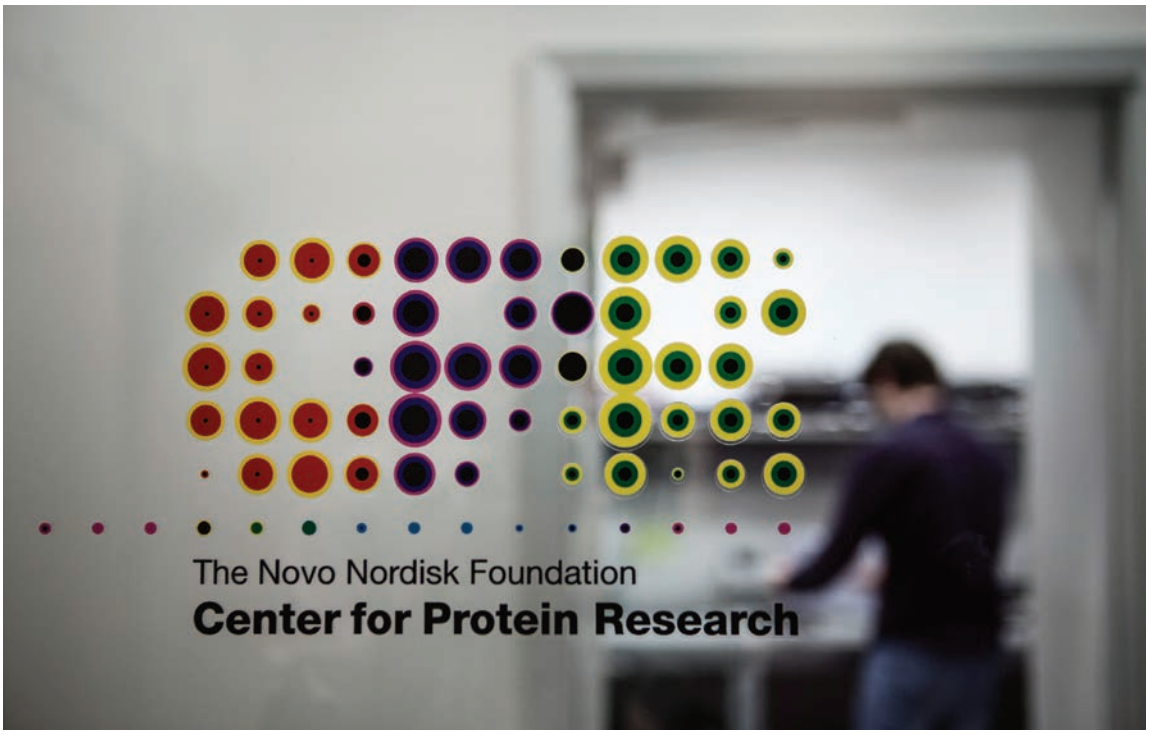
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