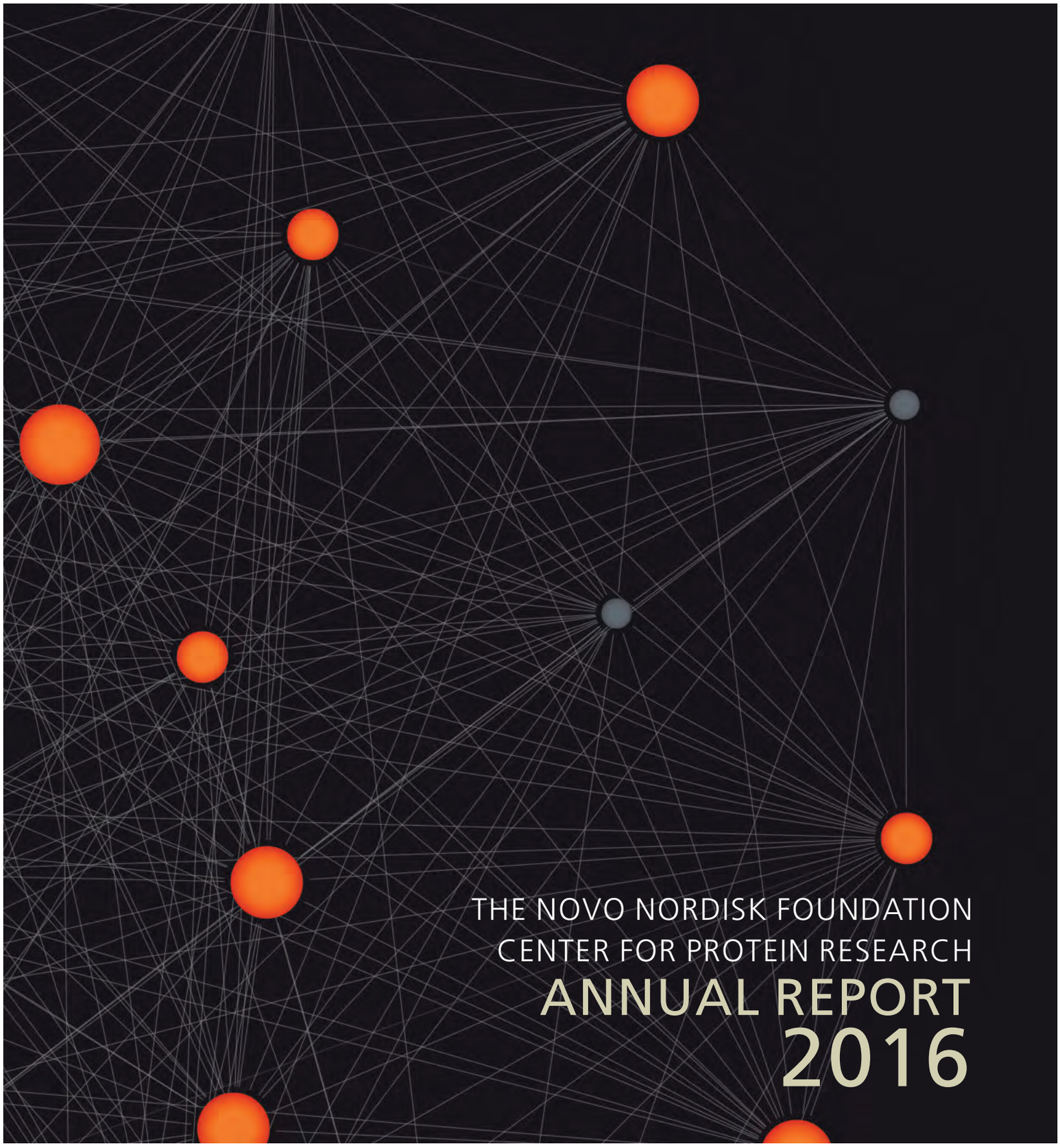
The Novo Nordisk Foundation
Center for Protein Research

UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES



THE NOVO NORDISK FOUNDATION CENTER FOR PROTEIN RESEARCH
ANNUAL REPORT 2016

NOVO NORDISK FOUNDATION CENTER FOR PROTEIN RESEARCH

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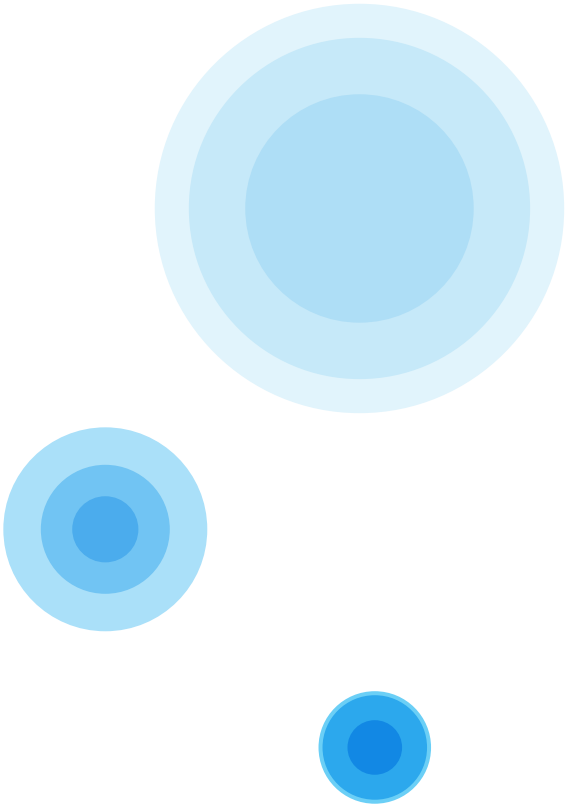


Front cover shows a network of RNA-binding proteins that are regulated by arginine methylation (Larsen *et al*., *Science Signaling*, 2016) .

CONTENTS

FROM THE MANAGEMENT

Jiri Lukas, Executive Director

2016 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 CPR's traditional scientific and technological strongholds, while at the same time expanding into new areas (see Figure 1 for selected milestones). Last, but not least, we have been very successful in attracting external funds for our research – in 2016 this amounted to 120 million DKK (16 million EUR).

CPR transforms internal synergy to flagship projects

A long-time goal for CPR has been to develop CPR flagship projects: highly original, long-term scientific endeavors that address outstanding biomedical issues and uniquely combine our technological and intellectual expertise to lead the world. In 2016, three initiatives have matured into flagship projects. First, clinical proteomics combines high-end mass spectrometry with advanced informatics, big data management and unique access to electronic Danish patient records. Second, our holistic approach to catalogue protein pathways that orchestrate genome integrity maintenance comprises a synergy between a multitude of large-scale proteomic and genetic screens coupled with in-depth mechanistic explorations. Third, our pioneering structural and functional approaches to understand how enzymes interact with their substrates combine unique and original expertise generated across CPR and they have matured to complex projects that give us major competitive advantages. These flagship initiatives are perfectly aligned with our objective to pursue protein-based precision medicine and will at the same time inevitably support our efforts to develop complete protein scientists and become an unmatched global partner in protein research.

CPR expanded with cryo-EM

Another milestone has been to integrate advanced cryo-electron microscopy (cryo-EM) to CPR research. In 2016, Research Director Guillermo Montoya secured a 60 million DKK (7.8 million EUR) grant from the Novo Nordisk Foundation to establish cryo-EM. Besides purchasing the latest generation of cryo-EM equipment, the

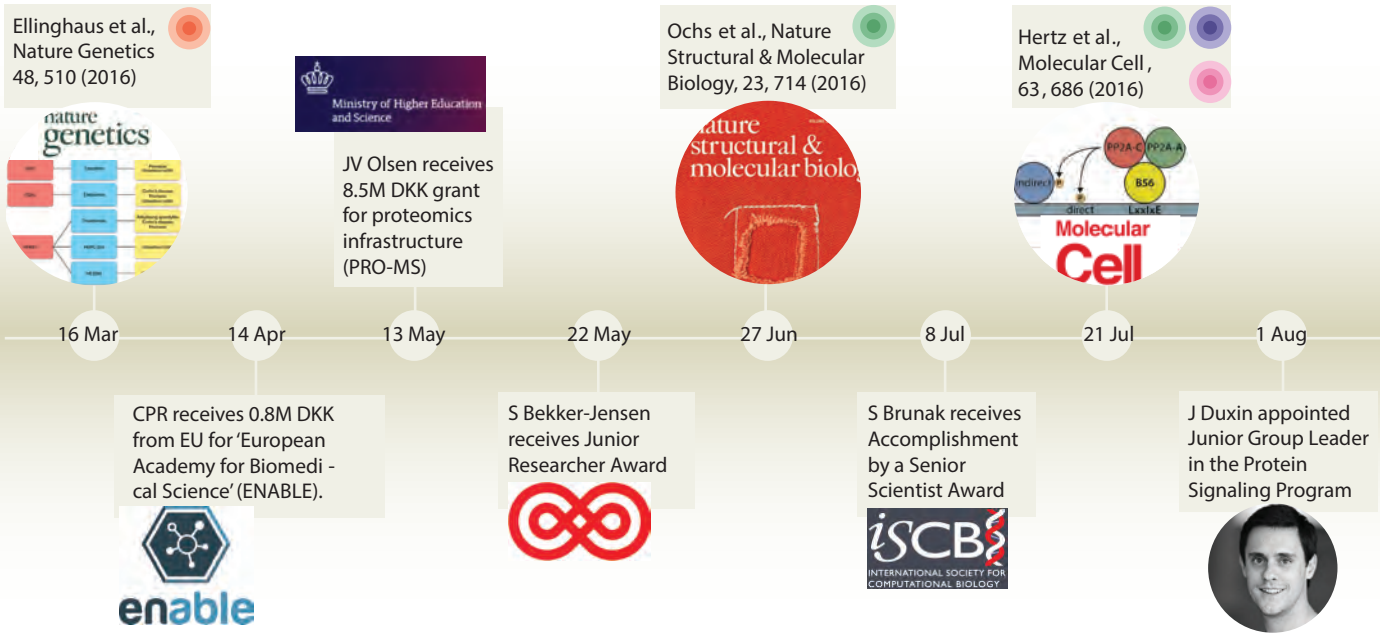


Figure 1 | Selected 2016 key milestones and achievements.

grant will allow Guillermo to expand his Program by a junior group. The operation of the cryo-EM unit will be a joint venture between CPR and Center for Integrated Microscopy (CFIM), which will further strengthen and expand our outreach to the Faculty of Health and Medical Sciences.

CPR's first junior group leader

We continuously strive to attract top talents to CPR to complement our world-leading team of scientists. In 2016, Julien Duxin joined the Protein Signaling Program as junior group leader. Julien made a stellar postdoc at Harvard Medical School in Johannes Walter's lab and we are extremely happy that he chose CPR to pursue his independent research. Julien's group will focus on replication-coupled repair of DNA-protein crosslinks, a genome maintenance mechanism with high medical relevance and strong complementarity both to the Protein Signaling Program and indeed the entire CPR. Julien's talent, scientific insight, and technological expertise are bound to be a huge asset not just for CPR, but for the Copenhagen chromosome biology community.

Home-grown CPR scientists take on prestigious independent positions

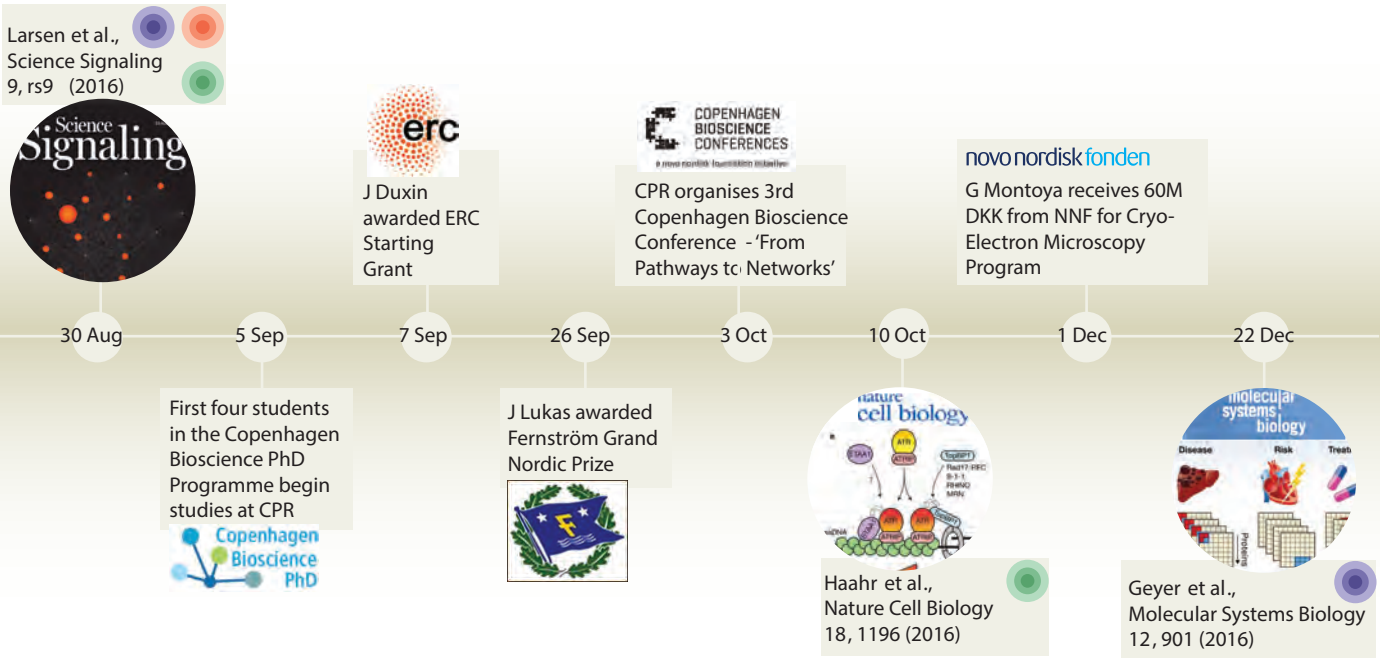
An important goal for CPR is to 'grow' future scientific leaders that can successfully compete outside CPR. This includes supplying some of our best researchers as the next generation of scientific leaders in the Faculty of Health and Medical Sciences, which we regard as one of the strongest ways CPR can contribute to develop the good name of our University. The first 'home-grown' ambassador of this kind was Alicia Lundby in 2015 (group leader, Department of Biomedical Science) and she was joined in 2016 by Simon Bekker-Jensen (group leader, Center for Healthy Aging) and Luis Toledo (group leader, Center for Chromosome Stability). Further highlighting the quality of the work they performed in CPR, all three were awarded prestigious grants and awards to start their independent groups, including a Sapere Aude grant from Danish Council of Independent Research, a Lundbeck Foundation Fellowship and a European Research Council starting grant.

International PhD program successfully kicked off

The launch of an international PhD recruitment program has been a central part of our strategy to build the identity of CPR and 2016 saw the arrival of the first four PhD students selected in the framework of the new Copenhagen Bioscience PhD Program sponsored by Novo Nordisk Foundation. We are grateful to the Novo Nordisk Foundation for this initiative, first of its kind in Denmark, which aligns CPR with some of the most renowned research hubs by competing for the brightest minds and young talents all around the world.

Third Copenhagen Bioscience Conference in Protein Signaling

A true highlight in 2016 was the third 'Protein Signaling' conference organized by CPR as part of the Copenhagen Bioscience Conference series, with generous logistical and financial support from



Novo Nordisk Foundation. Like the conferences in 2012 and 2014, this was an extremely successful and inspiring interdisciplinary meeting. It continues to be our ambition to develop the conference series into a hotspot for world-leading scientists who would find it essential to come to Copenhagen on a regular basis to discuss how to advance the research field of disease-related protein signaling on the world-wide scale.

Scientific Advisory Board strengthened in structural biology

All of the mentioned achievements could not have been obtained without continuous support and guidance from our Scientific Advisory Board (SAB). In 2016, long time SAB member Paul Nissen decided to step down and we want to thank Paul for all he has done for CPR over the years. We managed to persuade Christoph Müller, head of Structural and Computational Unit, EMBL (Germany), to join our SAB from 2017, which will be a great asset for CPR.

Scientific output

The main aspect of our achievements in 2016 is the remarkable scientific productivity of CPR. Scientists at CPR continue to publish outstanding results in high-impact journals and present their research in effective and engaging ways. In 2016, CPR researchers published 87 scientific papers of which a remarkable 28 papers were published in high impact journals (impact factor > 10), including the most prestigious journals such as Nature and Science and their sister journals.

The four CPR Programs achieved notable success in areas spanning from technology development over basic biological processes to applications in a clinical context. Some of the highest impact discoveries include: proof of concept for the clinical proteomics pipeline with accurate quantification of thousands of plasma proteins from just a finger prick of blood; analysis of time-ordered co-morbidities over periods of more than 20 years; integration of microbiome data in the study of insulin sensitivity; development of an app (Cytoscape) that provides easy access to protein associations from the STRING database enabling more advanced visualization and analyses of large protein networks; identification of the ETAA1 protein as a novel activator of the cellular defense against DNA replication stress, which brings us closer to understanding some of the main cell-fate decisions during cancer development; new and unorthodox insight into how proteins regulate the fidelity of repair of damages to the human genome; and the revolutionary discovery of how protein phosphatases recognize their substrates.

In addition to these highlighted discoveries, we are proud of our highly integrated scientific environment where researchers at all levels benefit from mutual synergy. We strive to transform internal synergy into longterm CPR flagship projects that address outstanding biomedical issues and uniquely combine our technological and intellectual expertise to lead the world.

Funding secured in 2016

Our scientists have succeeded in securing funding from many different sources in 2016. As already mentioned, NNF granted 60 million DKK (8 million EUR) to Research Director Guillermo Montoya to establish cryo-EM at CPR. A 1.9 million DKK (0.3 million EUR) was secured by the Brunak group (Disease Systems Biology Program) for the Horizon 2020 project 'ROADMAP'. Julian Duxin (Protein Signaling Program) received a 11.2 million DKK (1.5 million EUR) European Research Council (ERC) Starting Grant to investigate mechanisms of DNA-protein cross-link repair in S phase. Last but not least, Jesper V Olsen (Proteomics Program) was granted 8.5 million DKK (1.1 million EUR) for the grant 'Danish National Mass Spectrometry Platform for Functional Proteomics' from The Danish Agency for Institutions and Educational Grants (Ministry of Higher Education and Science). The grant is part of the national PRO-MS consortium that is supported by 40 million DKK (5.3 million EUR) to establish a national research infrastructure on mass spectrometry for functional protein research and proteomics. We look back on our achievements in 2016 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.

Jesper V. Olsen
Vice Director

Peter Dyrsting
Head of Administration and Finance

Jiri Lukas
Executive Director

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 Foundation Center for Protein Research (CPR) at the University of Copenhagen (UCPH), Faculty of Health and Medical Sciences continues to conduct protein research with an unprecedented depth and complexity, ranging from fundamental aspects of protein structure and biological function to a direct outreach to translational medicine. I am delighted about CPR's progress in 2016, where its strongholds in protein research in health and disease were further consolidated by groundbreaking discoveries and successful ventures into new scientific, technological and medical areas.

CPR retains its ability to attract and support young as well as established scientists that publish papers in top scientific journals and successfully compete for the most prestigious research awards and prizes. I am also pleased to see that in 2016, two excellent young scientists from CPR decided to pursue their independent research careers in our Faculty. This fulfils our expectations to the centers of excellence,and CPR is pioneering the concept of nurturing young scientists.

In sum, I am very impressed and proud of the entire CPR team for their many advances and achievements. CPR has proved again that it is a world leading hub for protein research that is deeply rooted in our University and very significantly contributes to reinforce Copenhagen's position as an international city of knowledge.

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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 generous support from the Novo Nordisk Foundation (NNF) and the Faculty of Health and Medical Sciences 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 is 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 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 NNF and UCPH, CPR has

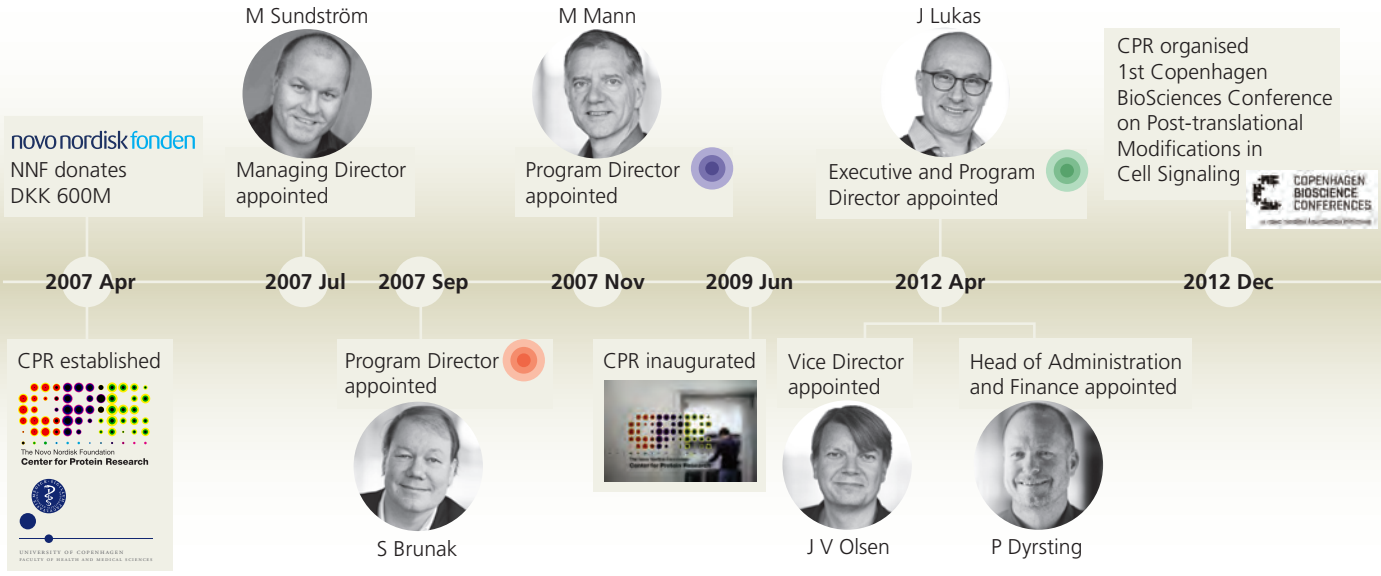


Figure 2 | Selected milestones during 2007-2015.

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

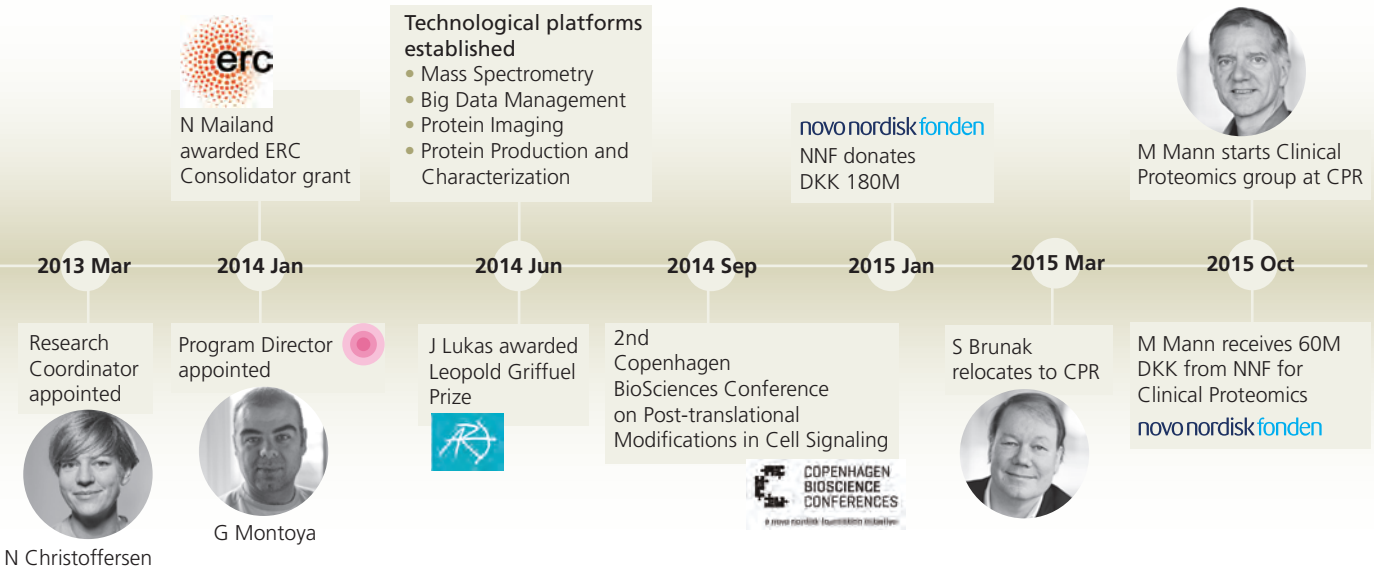
CPR is proud to have gathered a team of world-leading scientists to direct its four Programs in Proteomics (Matthias Mann), Protein Structure and Function (Guillermo Montoya), Protein Signaling (Jiri Lukas), and Disease Systems Biology (Søren Brunak), together with extremely talented young group leaders 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 Copenhagen Bioscience PhD recruitment program and, for instance, helped initiate the 'thesis advisory committees' with our colleagues from DanStem, 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 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, many

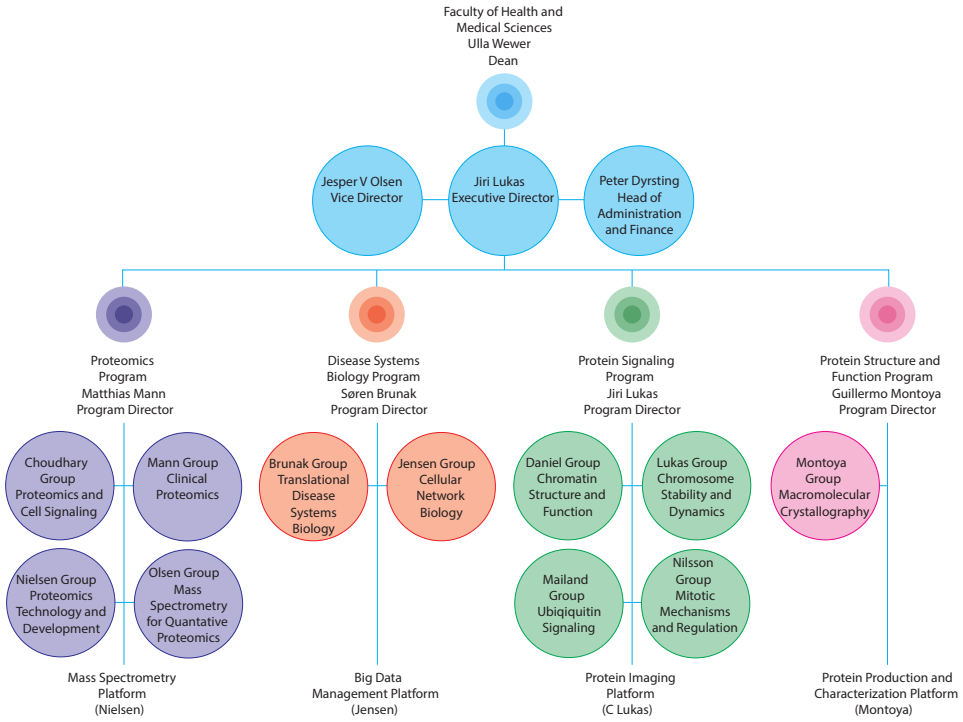


Figure 3 | CPR consists of four highly integrated scientific programs and associated technology platforms.

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 2016

While the details of CPR's achievements in 2016 are described throughout this Annual Report and selected highlights are illustrated in Figure 1, three main advances stand out.

The first is the expansion of CPR's Programs and Platforms with cryo-electron microscopy (cryo-EM). The 60 million DKK (7.8 million EUR) grant donated by the Novo Nordisk Foundation will enable Research Director Guillermo Montoya to establish cryo-EM at CPR and expand his Program with a junior research group that will also mediate the cryo-EM technology across CPR and beyond to the growing Copenhagen-based bioscience community. The addition of cryo-EM to CPR's technological portfolio consolidates our position at the forefront of protein research. At the same time, the operation of the cryo-EM unit will be a joint venture between CPR and Center for Integrated Microscopy (CFIM) at Faculty of Health and Medical Sciences and we are excited about this opportunity to strengthen our ties to the Faculty.

The second achievement is the impact made by CPR's scientific discoveries and innovations in 2016 as demonstrated by the 87 papers published, mostly in very high-impact journals.

The third is our continued effort to train young scientists to become future scientific leaders that can successfully compete outside CPR. In 2016, Simon Bekker-Jensen and Luis Toledo moved on to become group leaders at our Faculty, at Center for Healthy Aging and Center for Chromosome Stability, respectively.

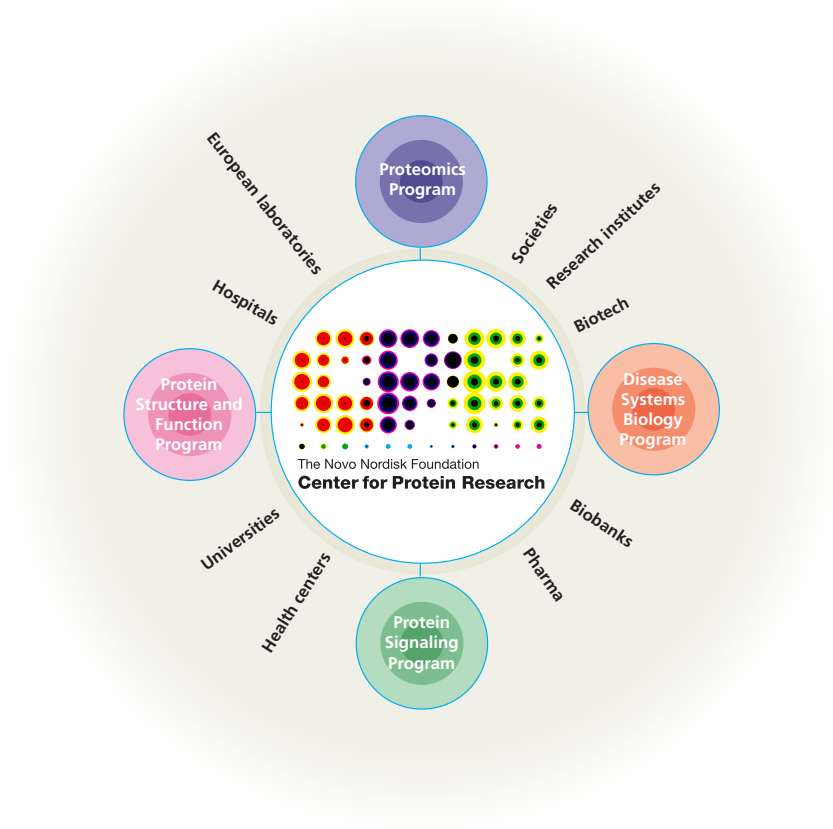


Figure 4 | CPR is a highly integrated research center, with both internal and external stakeholders.

GOVERNANCE AND ORGANIZATION

In 2016, CPR's four research Programs and their corresponding technology Platforms, set up in the earlier years of CPR's existence, were further consolidated and integrated. The Programs and the Platforms are still developing, and the most important developments are highlighted here.

In 2016, an additional research group was added to the Protein Signaling Program with the recruitment of Julien Duxin who joined CPR in August 2016 as a junior group leader. Julien Duxin comes from an impressive postdoc at Harvard Medical School and at CPR he will continue his research focus on protein pathways that protect the integrity of human genomes against metabolic assaults that generate DNA-protein crosslinks. With its five research groups and the Protein Imaging Platform, the Protein Signaling Program has a total of 37 staff members.

In the Protein Structure and Function Program, Research Director Guillermo Montoya secured a 60 million DKK (7.8 million EUR) grant from the Novo Nordisk Foundation to establish cryo-electron microscopy (cryo-EM). As mentioned on previous pages, this grant will allow CPR to purchase the latest generation of cryo-EM equipment and expand the Program with a junior research group. The operation of the cryo-EM unit will be a joint venture between CPR and Center for Integrated Microscopy (CFIM) at Faculty of Health and Medical Sciences and Guillermo Montoya's Program will help mediate the cryo-EM technology across CPR and beyond. The microscope installation and the recruitments are expected to be completed during 2017. By the end of 2016, the Protein Structure and Function Program is hosting 23 staff members.

In the Proteomics Program, Matthias Mann's Clinical Proteomics group, which was established only last year, has expanded to include seven staff members at the end of 2016. An important addition to the Program in 2016 was the recruitment of mass spectrometry specialist Simone Schopper to the Mass Spectrometry Platform. With this addition, the Platform is in an even stronger position to offer state-of-the-art proteomics analysis at the highest international level to its collaborators at CPR as well as externally. By the end of 2016, the Proteomics Program employs 36 staff members and is expected to add additional team members in 2017 due to the further expansion of Matthias Mann's group and a growing number of externally funded research projects.

Further to his full-time relocation to CPR in 2015, Søren Brunak's research group 'Translational Disease Systems Biology' is now running full speed at CPR. The Disease Systems Biology Program is continually expanding due to new external funding, primarily EU projects as for instance the ROADMAP consortium where the Program is represented with several researchers. At the end of 2016, the Disease Systems Biology Program is hosting 37 staff members and it is expected to expand further in 2017.

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 and has established numerous successful collaborations with research groups at other departments at the Faculty. CPR sees the embedment in the University as win-win, as our state-of-the-art technology platforms, in particular, make CPR an attractive collaboration partner for researchers from other parts of the Faculty and, in turn, CPR researchers gain crucial biological and medical knowledge as well as exclusive access to state-of-the-art facilities and patient samples.

As a way of further strengthening our involvement with the Faculty we supply some of our best researchers as the next generation of scientific leaders in the Faculty of Health and Medical Sciences. These future leaders are a precious asset to CPR, which we are proud of and which we regard as one of the strongest ways we can contribute to develop the good name of our University. The first 'home-grown' ambassador of this kind was Alicia Lundby in 2015 (now group leader in Department of Biomedical Science) and she was joined in 2016 by Simon Bekker-Jensen (group leader, Center for Healthy Aging) and Luis Toledo (group leader, Center for Chromosome Stability).

"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

Another means by which we contribute to the continuing integration with the Faculty is by organizing Master's and PhD courses in protein technologies. In 2016, CPR converted our previous pre-graduate course in protein technologies to a stand-alone international summer school entitled 'Advanced Methods for the Analysis of Protein Disease Mechanisms', which will now expectedly take place every year in August and September. This course complements the Faculty teaching portfolio by a unique combination of e-lectures, real-time online access by students to their teachers, and meticulously designed practical exercises spanning all the major analytical areas of protein research covered at CPR. In 2016, the summer school hosted 23 Danish

and international students and was highly rated by the participants.

Last but not least, the monthly CPR Seminars Series is open to all researchers and students at the Faculty. The seminars are advertised to other interested parties at the University and elsewhere. The seminar speakers are world-leading experts in areas of protein research that are invited to present their latest research and actively interact with CPR's students, postdocs, and group leaders.

STAFF AND RECRUITMENT

At the end of 2016, CPR employs 147 staff members from 32 different countries compared to 124 at the end of 2015. Of the 147 staff members, 45 were recruited in 2016. In the same period, 22 staff members left CPR, of which 16 had completed PhD or postdoctoral fellowships with us.

CPR employees fall in three categories: scientific personnel, research support, and administrative support, with scientific personnel constituting 71 % in 2016. In 2016, the number of scientific staff increased from 89 to 110, primarily resulting from the launch of several new research projects, the establishment of the Duxin group and the continued progression of the Mann and Brunak groups. In addition, the research support staff, which also includes technological platform personnel, increased from 23 to 28 staff members in 2016.

At CPR we have succeeded to maintain an overall gender balance of approximately 50/50 (see Figure 5). At the management level we have one female leader, Professor Claudia Lukas (Protein Imaging Platform), among 14 managers at CPR. See Figure 5 for an overview of staff composition at CPR in 2016.

CENTER MANAGEMENT

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

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

"We are proud that we have achieved and maintained a 50/50 gender balance across CPR and we strive for a similar balance at management level. To do this, we have implemented measures to help us attract a more balanced field of applicants when we search for highly qualified group leaders."

PETER DYRSTING,
HEAD OF ADMINISTRATION
AND FINANCE

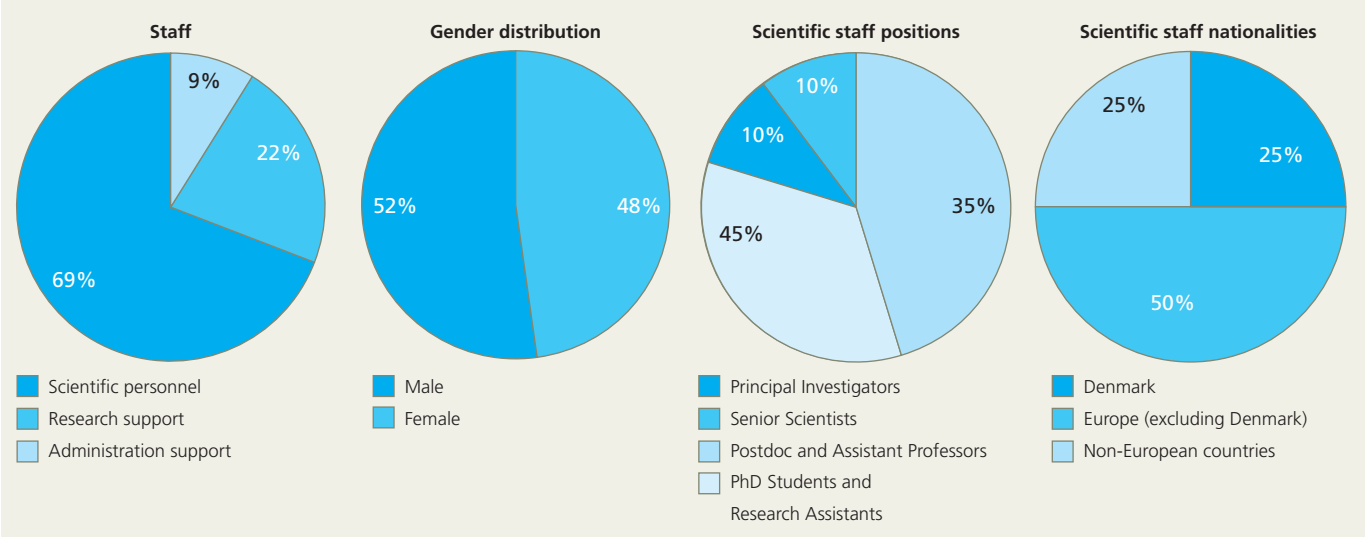


Figure 5 | Staff composition in 2016.

In addition, Group Leader meetings take place 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 CPR's Student and Postdoc Association (SPA) as well as CPR specialists on specific topics the opportunity to come up with suggestions and give their opinion on important strategic decisions.

"SAB remains impressed with both the scientific development of the center and its productivity. It is clear that the management of the CPR constantly strives to generate synergy that makes the institute more than the sum of its parts."

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

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 an overview of 2016 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.

In 2016, SAB member Poul Nissen stepped down and he will be replaced from 2017 by Christoph Müller, Head of Structural and Computational Unit at EMBL, Heidelberg. Christoph Müller combines strong expertise in cryo-EM, X-ray crystallography and advanced biophysical and biochemical approaches with his own research on protein-DNA interactions and chromatin-modifying complexes, which are all relevant areas for many CPR research projects. In addition, he has ample experience with running research groups and state-of-the-art facilities.

2016 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, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 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.*

"I am extremely impressed with the accomplishments of SPA and in 2016 especially for succeeding to persuade Bruce Stillman, Director of Cold Spring Harbor Laboratories and founder of the DNA replication field to spend a few days in CPR!".

JIRI LUKAS

STUDENT AND POSTDOC ASSOCIATION

CPR's Student and Postdoc Association (SPA) has become a strong voice that strives to strengthen the social and scientific networks amongst junior researchers at CPR. SPA representatives have a regular slot at CPR Group Leader meetings and many changes and improvements have been implemented as a result of SPA's ability to utilise this opportunity for discussion with and feedback from the group leaders.

In 2016, SPA continued to promote social interactions, invite prominent scientists as speakers in the CPR Seminar Series, arrange career days for junior researchers and represent its members in center-related issues. SPA's annual scientific writing workshop was enthusiastically received by the participants and is now an annual event.

Also in 2016, SPA succeeded to invite and host world-leading scientist Bruce Stillman, director of Cold Spring Harbor Laboratories, who did not only give a spectacular seminar, but also indulged questions and informal discussions with young scientists from CPR in several social outings arranged by SPA.

The second SPA retreat held in September 2016 featuring vivid interactions and plentiful scientific feedback. The retreat has become an established annual event in the social and scientific life at CPR.

At the Faculty of Health and Medical Sciences, SPA is part of the career development platform "CaOp" with junior staff from DanStem, Department of Cellular and Molecular Medicine (ICMM) and Department of Biomedical Sciences (BMI). With the support of CPR management, SPA is actively promoting mobility and expertise of junior researchers by co-organizing a Horizon2020-funded symposia series under the project name 'ENABLE' (European Academy for Biomedical Science). The initiative seeks to promote excellence in the biomedical sciences in Europe, strengthen scientific careers, and bring biomedicine closer to society.

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. Support for CPR's research groups covers a wide range of tasks, including purchasing, meeting organization and general support for group members and running of laboratories. In addition, group leaders and grant recipients receive support for communication tasks, registration of publications, project coordination, health and safety requirements, recruitment, and finances. In addition to these areas, support for CPR's senior management includes strategic planning and counseling. The administration group is, in turn, supported at both Faculty and University level, 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 RESEARCH DIRECTOR

MATTHIAS MANN

"We continuously 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.

Organization

The Proteomics 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. The Program 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 Mass Spectrometry Platform, and myself. My group, the Clinical Proteomics group, was established in 2015 and has now grown to seven members.

Technological advances

Exemplifying technological advances, Jesper V Olsen's group achieved unparalleled depth of proteome coverage, with nearly all expressed genes identified as proteins in as little as one day (*manuscript in preparation*). At this depth of analysis, many protein modifications were apparent even without enrichment. Using their advanced pipeline to characterize PTMs, especially phosphorylation, they revealed the molecular mechanisms explaining functional selectivity of different growth factors activating the same receptor tyrosine kinase on the plasma membrane of human cells (Francavilla et al., *Nature Structural and Molecular Biology*, 2016). They also reported a new technology to identify more than 10,000 neuropeptides (Secher et al., *Nature Communications*, 2016).

The initial focus of the Clinical Proteomics group has been on technology development that has brought about a breakthrough in the preparation and robust analysis of the blood proteome (plasma or serum). This technology was then applied to a first clinical study – the analysis of the plasma proteome during weight loss and weight maintenance (Geyer et al., *Molecular Systems Biology*, 2016).

Research highlights

The Nielsen group developed two novel proteomics techniques for comprehensive characterization of arginine mono-methylation and ADP-ribosylation, respectively (Larsen et al., *Science Signaling*, 2016; Martello et al., *Nature Communications*, 2016). Using these innovative methods, they elucidated the wide-spread occurrence and functional role that these emerging PTMs play in mammalian cells and tissue and uncovered novel biological aspects not previously associated with these modifications.

Using SILAC-based proteomics, the Choudhary group quantified systems-level signaling by pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) (Wagner et al., *EMBO Journal*, 2016). In addition to providing a panoramic view of TNF-α signaling, the work gave mechanistic insights into the assembly of TNF-α signaling complexes by identifying SPATA2 as a novel component of this signaling pathway. SPATA2 recruits deubiquitylase CYLD to the receptor signaling complex, which is necessary for TNF-α-induced necroptosis. The work exemplifies the usefulness of quantitative proteomics in discovering novel functional regulators even in the most extensively studied signaling pathways.

International standing and collaborations

Internally, the Proteomics Program has established strong collaborations with virtually every research group at CPR, helping to give them a crucial competitive edge in research and in attracting grants. Cooperation in the Copenhagen area includes other NNF centers, Eske Willerslev's group at Center for GeoGenetics (University of Copenhagen), 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 University of Cambridge and many other prestigious universities worldwide.

"We combine quantitative proteomics with cell and molecular biology approaches to understand the architecture and functions of protein signaling networks."

PROTEOMICS AND CELL SIGNALING

CHOUDHARY GROUP

Professor
Chunaram Choudhary (Group Leader)

Associate Professor
Brian T. Weinert

Postdocs
Thomas Wild
Magdalena Budzowska
Rajat Gupta
Takeo Narita
Balaji Srinivasan

PhD Students
Shankha Satpathy

Research Assistant
Bo Karbech Hansen

Technicians
Elina Maskey
Rebeca Soria Romero

INTERNAL COLLABORATORS

- **Jakob Nilsson** (Nilsson group, Protein Signaling Program) functional analysis of ACP/C-associated E2 ubiquitin ligase enzymes.
- **Jeremy A Daniel** (Daniel group, Protein Signaling Program) analysis of PTIP functions.
- **Jiri Lukas** and **Niels Mailand** (Lukas group, Mailand group, Protein Signaling Program) characterization of novel DNA damage repair factors.

EXTERNAL COLLABORATORS

Michael Lisby (Center for Chromosome Stability, University of Copenhagen (UCPH), Denmark) analysis of Mte1 in DNA recombination and repair.

Stephen Cohen (Department of Cellular and Molecular Medicine, UCPH, Denmark) investigation of USP9X function in hippo signaling.

Frank Buchholz (University of Dresden, Germany) identification of novel stem cell pluripotency regulating factors.

2016

Challenges and research aims

In recent years, mass spectrometry (MS)-based proteomic studies have identified a large number of acetylation and ubiquitylation sites. The next challenge is to find out how specific modification sites are dynamically regulated in response to signaling cues, what is the stoichiometry of modification sites, and how protein interactions in signaling pathways are re-modeled in response to activating signals. Our group combines MS-based technologies with cell and molecular biology approaches to tackle these questions. Our goal is to systematically generate large datasets for uncovering novel protein functions. From unbiased proteomic screens, we investigate functions of selected proteins and PTMs to discover their functions and to gain insight into their molecular mechanisms. Our strengths are ideally complemented by the expertise of our in-house collaborators, providing us with a strong competitive edge for pursuing interdisciplinary and challenging projects in this area.

STRATEGIC GOALS

- To understand the stoichiometry of lysine acetylation in cells and tissue and to understand the evolutionary relevance of mitochondrial sirtuins
- To systematically investigate the networks of tumor necrosis factor alpha receptor signaling

Achievements

Cytokine tumor necrosis factor alpha (TNF-α) is a major regulator of innate immune and pro-inflammatory responses. We used quantitative mass spectrometry to understand the composition of the TNF-α receptor-associated signaling complexes (TNF-RSC) and the architecture of the downstream signaling networks. We simultaneously investigated the dynamics of ligand-activated TNF-RSC and of the downstream phosphorylation and ubiquitylation. We showed that TNF-α stimulation induces widespread changes in protein phosphorylation and ubiquitylation. Temporal analysis of the TNF-RSC composition identified SPATA2 as a novel component of the TNF-RSC. We showed that the predicted PUB domain in the N-terminus of SPATA2 interacts with the USP domain of CYLD, whereas the C-terminus of SPATA2 interacts with HOIP (Figure 6). SPATA2 is essential for recruitment of CYLD to the TNF-RSC. Down regulation of SPATA2 augments transcriptional activation of NF-κB and inhibits TNF-α-induced necroptosis, pointing to an important function of SPATA2 in modulating the outcomes of TNF-α signaling. This work was published in *EMBO Journal* (Wagner et al., *EMBO Journal*, 2016).

In a close collaboration with the Nilsson group at CPR, we investigated the functions of E2 ubiquitin-conjugating enzymes and K11 ubiquitin linkages in mitosis. The anaphase-promoting complex/cyclosome (APC/C) and the spindle assembly checkpoint (SAC), which inhibits the APC/C, are essential determinants of mitotic timing and faithful division of the genetic material. We showed that APC/C activity in human cells is tuned by the combinatorial use of three E2s, namely UBE2C, UBE2S, and UBE2D. Genetic ablation of UBE2C and UBE2S, individually or in combination, leads to discriminative reduction in APC/C function and sensitizes cells to UBE2D depletion. Reduction of APC/C activity results in loss of switch-like metaphase-to-anaphase transition and, strikingly, renders cells insensitive to chemical inhibition of MPS1 and genetic ablation of MAD2, both of which are essential for the SAC. These results, which were published in *Cell Reports* (Wild et al., *Cell Reports*, 2016), provide new insights into the regulation of APC/C activity and demonstrate that the essentiality of the SAC is imposed by the strength of the APC/C.

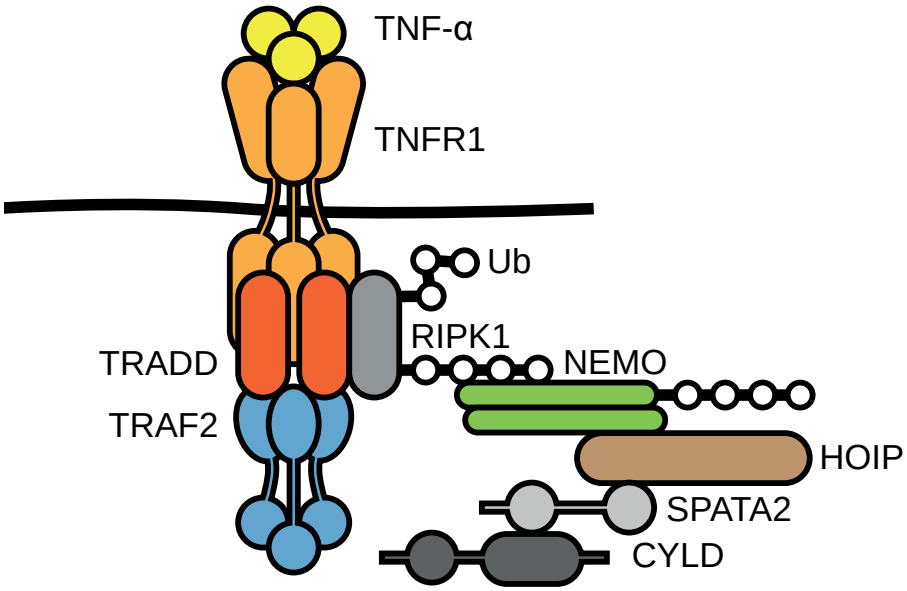


Figure 6 | Model for SPATA2 function in TNF-α signaling. SPATA2 interacts with CYLD and HOIP under steady-state conditions and TNF-α stimulation results in their recruitment to TNF-RSC. SPATA2-dependent recruitment of CYLD impacts on the activation of TNF-α-induced NK-kB signaling and necroptosis (Wagner et al., *EMBO Journal*, 2016).

"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)

Associate Professor
Atul Deshmukh

Postdoc
Niels H Skotte

PhD Students
Lili Niu
Philipp Geyer
Nicolai Jacob Wewer Albrechtsen

Research Assistant
Helle Baltzer Hattel

Also featured in group picture:
Camilla Johansson (Program Coordinator, 2017)
Andreas Mund (Assistant Professor, 2017)
Alberto Santos Delgado (Postdoc, 2017)
Fabian Coscia (Postdoc, 2017)
Florian Meyer (PhD Student, Max Planck Institute of Biochemistry, Munich, Germany)
Sophia Doll (PhD Student, Max Planck Institute of Biochemistry, Munich, Germany)

INTERNAL COLLABORATORS

Niels Mailand (Mailand group, Protein Signaling Program) DNA damage response.

Søren Brunak (Brunak group, Disease Systems Biology Program) establishing a joint effort on big data analysis of relevance for precision medicine based on clinical proteomics.

EXTERNAL COLLABORATORS

Jens Juul Holst (Novo Nordisk Foundation (NNF) Center for Basic Metabolic Research, University of Copenhagen (UCPH), Denmark) plasma proteomics in metabolic disease.

Juleen Zierath (NNF Center for Basic Metabolic Research, UCPH, Denmark) skeletal muscle secretome.

Bente Klaarlund Pedersen (Rigshospitalet, Denmark) plasma proteomics in the context of exercise.

Novo Nordisk A/S (Denmark) discovery of secreted proteins with potential anti-diabetic properties.

2016

Challenges and research aims

Measuring all the proteins in human blood could in principle provide a way to characterize the health or disease state of any person and any disease. However, analyzing the plasma proteome is technologically extremely challenging. This is because blood has a very high concentration of proteins but most of the protein mass is due to just a few, extremely abundant proteins such as serum albumin. Most proteins of interest are a million to a billion fold less abundant, making them very difficult to detect and quantify. As a result, plasma proteomics had been largely abandoned as a mainstream research direction. Enabled by our Novo Nordisk Foundation grant for Clinical Proteomics, we have revisited plasma proteomics and have already made great technological advances. In 2016, we have developed a robust plasma proteomics sample preparation pipeline, which is implemented on a robotic platform. We are on schedule to double the depth of protein identification and to apply this technology to many small scale clinical studies in the near future. Based on these results, it will be very exciting to see what the information content of the plasma proteome actually is.

STRATEGIC GOALS

- To develop the technology necessary to measure the plasma proteome to a depth of 1000 proteins at high quantitative accuracy at a throughput of 100 plasma proteomes per day
- To apply plasma proteomics profiling to a large range of clinical studies to discover protein patterns characteristic of health and disease states
- To use this information in early diagnosis and classification of the metabolic syndrome and other disease states as well as to guide lifestyle or medical interventions

Achievements

In 2016, we demonstrated a robust and scalable sample preparation technology for the human plasma proteome. We characterized hundreds of samples with the goal of determining sources of variability in the protein levels due to the workflow, within the same person over time and between individuals. Proof of principle of this technology was published in 2016 in the open access journal *Cell Systems* (Geyer et al., *Cell Systems*, 2016). The paper, entitled 'Plasma proteome profiling to assess human health and disease' has raised quite a bit of interest.

At the end of the year, we followed this up by our first clinical study using plasma proteome profiling. This paper, published as 'Proteomics reveals the effects of sustained weight loss on the human plasma proteome' (Geyer et al., *Molecular Systems Biology*, 2016), analyzed more than 1200 plasma proteomes of a cohort of morbidly obese individuals (Figure 7). We found specific responses of proteins to weight loss and weight maintenance over a year. The data allowed us to define an inflammatory protein pattern and to quantify this dynamically in the study participants. We also investigated the relation of the plasma proteome to classical blood tests for the first time.

In our ongoing collaboration with Novo Nordisk A/S and Bente Klarlund Pedersen (Rigshospitalet, Denmark), we discovered a number of proteins that appear to be specifically secreted by human brown fat cells. Follow up on a number of these candidates revealed exciting phenotypes in a diabetic context. We are now expanding our collaboration into secreted peptides from brown cells. Also in collaboration with Bente Klarlund Pedersen, we have performed our first clinical study. Enrolled volunteers had to perform a bout of exercise and we obtained standard laboratory values and samples of body fluid proteomes. The aim of this study is to define a pattern of plasma protein changes reflecting physical exercise.

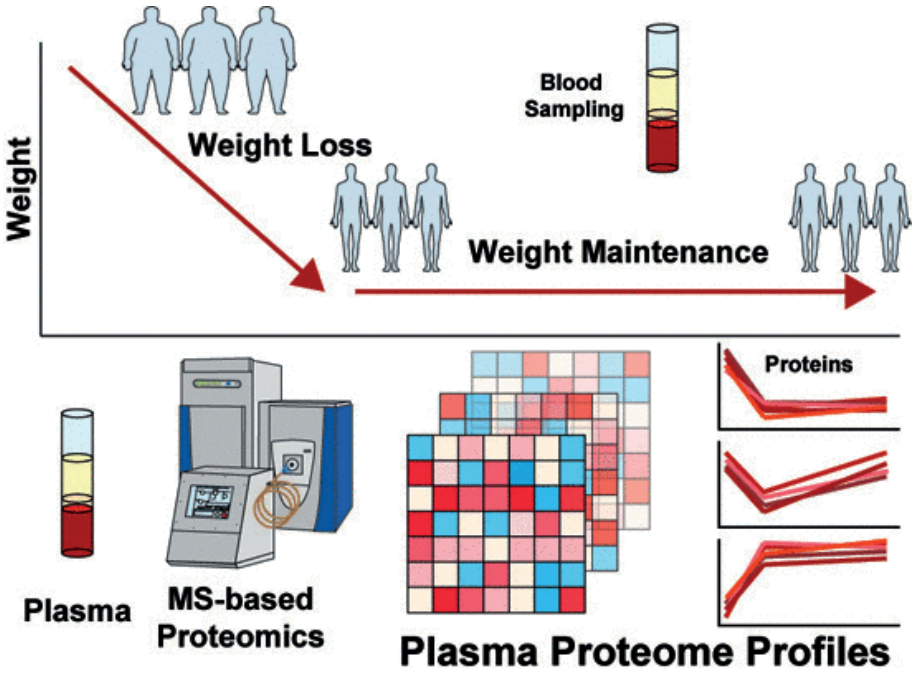


Figure 7 | Longitudinal plasma proteome profiling of 52 obese individuals during weight loss and maintenance reveals 93 significantly altered proteins, including panels correlating with inflammation and insulin resistance (Geyer et al., *Molecular Systems Biology,* 2016).

"We combine development of proteomic technologies with imaging and bioinformatics expertise at CPR to gain deeper understanding of how post-translational modifications globally impact cellular systems in mammalian cells."

PROTEOMICS TECHNOLOGY AND DEVELOPMENT

NIELSEN GROUP

Professor
Michael L Nielsen (Group Leader)

Postdocs
Ivo A Hendriks
Clifford Young
Niels H Skotte

PhD Students
Sara C Larsen
Meeli Mullari

Also featured in group picture:
Molly Lowndes (Postdoc, 2017)
Simone Schopper (Mass Spectrometry Specialist in the Mass Spectrometry Platform)

INTERNAL COLLABORATORS

Lars Juhl Jensen (Jensen group), Disease Systems Biology Program) bioinformatics analyses of proteomic data.

Niels Mailand (Mailand group, Protein Signaling Program) ubiquitylation and sumoylation.

Jakob Nilsson (Nilsson group, Protein Signaling Program) mitotic cell cycle regulators.

Jeremy A Daniel and **Julien Duxin** (Daniel group, Duxin group, Protein Signaling Program) DNA-binding proteins.

Jesper V Olsen (Olsen group, Proteomics Program) proteomic technology development.

Jiri Lukas (Lukas group, Protein Signaling Program) ADP-ribosylation.

EXTERNAL COLLABORATORS

Anders H Lund (Biotech Research and Innovation Center, University of Copenhagen (UCPH), Denmark) RNA-binding proteins.

Simon Bekker-Jensen (Center for Healthy Aging, UCPH, Denmark) RNA-binding proteins.

Javier Peña Diaz (Center for Healthy Aging, UCPH, Denmark) DNA damage.

Andre Nussenzweig (National Cancer Institute/ National Institutes of Health, US) protein pathways subverted in cancer.

Michael O Hottiger (University of Zurich, Switzerland) ADP-ribosylation.

Nick Lakin (University of Oxford, UK) histone ADP-ribosylation.

Tony Kouzarides (University of Cambridge, UK) histone PTMs.

Philip M Iannaccone (Northwestern University, US) intronic regulation of GLI1.

Manuel Stucki (University of Zurich, Switzerland) proteomic analysis in the DNA damage response.

Maria Christophorou (University of Edinburgh, UK) citrullination in human cells by the enzyme PADI4.

2016

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 post-translational modifications (PTMs) are currently amenable for analysis on a global scale. These include phosphorylation, ubiquitylation and acetylation. Developing technologies for 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.

STRATEGIC GOALS

- To establish proteomics methods for large-scale characterization of emerging PTMs such as SUMOylation and citrullination
- To develop novel methods for characterization of RNA- and DNA-binding protein regions using affinity-purification mass spectrometry (AP-MS) strategies
- To apply in-house methods to new mass spectrometry-based proteomics studies of tissue-specific ecto-ADP-ribosyltransferases

Achievements

In 2016, we developed and applied two novel proteomics techniques for comprehensive characterization of arginine methylation and ADP-ribosylation, respectively (Larsen et al., *Science Signaling*, 2016; Martello et al., *Nature Communications*, 2016). Using these imperative methods we have elucidated the wide-spread role these emerging PTMs play in human cells, while uncovering novel aspects not previously appreciated.

In the research article describing novel aspects related to arginine methylation, we uncover that arginine methylation is much more prevalent throughout the human proteome than previously anticipated and that specific arginine methylation sites regulate the localization and function of splicing and RNA transport factors. Using quantitative proteomics combined with RNA interference and high-throughput single-cell imaging, we specifically reveal that arginine methylation is involved in regulating two proteins participating in RNA processing and transport. Our analyses revealed that arginine methylation sites catalyzed by distinct arginine methyltransferases control the localization and RNA binding functions of the pre-mRNA splicing factor SRSF2 and the RNA-transporting activity of the protein HNRNPUL1 (Larsen et al., *Science Signaling*, 2016). The impact of our research story on characterization of arginine methylation in human was further emphasized as the story was selected as the cover image of *Science Signaling* (Figure 8).

We have additionally established a proteomic workflow for sensitive and unbiased analysis of endogenous ADP-ribosylation sites. This approach allows for the assessment of the role of ADP-ribosylation and ADP-ribosyltransferases in physiological and pathological states (Martello et al., *Nature Communications*, 2016).

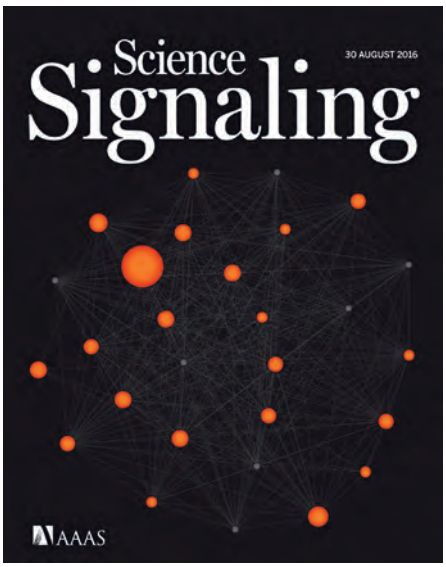


Figure 8 | A network of RNA-binding protein complexes that are regulated by arginine methylation, as featured on the cover of *Science Signaling*. The study identified and quantified more than 8000 unique arginine methylation sites and determined their dynamics. This is by far the largest number of identified arginine methylation sites in human cells reported so far and it significantly expands the current knowledge database (Larsen et al., *Science Signaling,* 2016).

"We want to address unsolved questions in cell signaling by leveraging our world-leading expertise in phosphoproteomics and quantitative mass spectrometry."

MASS SPECTROMETRY FOR QUANTITATIVE PROTEOMICS

OLSEN GROUP

Professor
Jesper V Olsen (Group Leader)

Associate Professor
Christian Dahl Kelstrup

Postdocs
Tanveer Singh Batth
Giulia Franciosa
Indranil Paul
Louise von Stechow
Magnus Erik Jakobsson

PhD Students
Anna-Kathrine Pedersen
Moreno Papetti
Rosa Rakownikow Jersie-Christensen
Alexander Hogrebe
Anamarija Pfeiffer

Research Assistant
Meaghan Mackie

Technicians
Dorte Breinholdt Bekker-Jensen

Also featured in group picture:
Ole Østergaard (affiliated group member, Statens Serum Institut)
Ana Martinez (visiting scientist, PhD Student, CNIO, Spain).

INTERNAL COLLABORATORS

Jiri Lukas (Lukas group, Protein Signaling Program) DDR and RPA interactome.

Jakob Nilsson (Nilsson group, Protein Signaling Program) PP2A interactome.

Michael L Nielsen (Nielsen group, Proteomics Program) proteomics technology.

Guillermo Montoya (Montoya group, Protein Structure and Function Program) MASTL and TLK2 phosphoproteomics.

Lars Juhl Jensen (Jensen Group, Disease Systems Biology Program) analysis of kinase-substrate relationships and protein interactions.

EXTERNAL COLLABORATORS

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

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

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

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

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

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

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

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

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

2016

Challenges and research aims

We are interested in understanding how different growth factors binding to the same receptor tyrosine kinase on the outside of cells activates differential signaling pathways and signals different cellular outcomes, a phenomenon known as functional selectivity. Developing and applying quantitative mass spectrometry-based proteomics technologies allows us to generate quantitative maps of global dynamic post-translational modifications, interactome and protein expression changes as a function of stimuli and stimulation time. These datasets form the basis of hypothesis generation that we test using various cell-based assays.

STRATEGIC GOALS

- To establish a powerful peptidomics workflow and apply it to global analysis of neuropeptides
- To characterize how cellular signaling processes mediated by phosphorylation and ubiquitylation differentially regulate cellular responses to biased ligand-induced receptor tyrosine kinase activation
- To develop mass spectrometric technology for comprehensive analysis of human proteomes and the major post-translational modifications without the need for specific enrichment methods

Achievements

We have developed and applied a novel streamlined analysis pipeline that lifts some of the haze surrounding the limited knowledge of the endogenous peptides that circulate within our bodies. Multiple challenges have so far restricted the number of endogenous peptides we can discover. A very big challenge originates from the fact that a major part of tissues and cells are made up of proteins that are constantly being synthesized and degraded in the body. Hence, a high number of detectable endogenous peptides simply represent fragments or remnants of proteins being turned over and undergoing recycling processes. So how do we know if a peptide is simply a recycling product or a novel form of insulin? To address this problem, we inhibited recycling of proteins through application of chemical inhibitors and a biophysical trick, where heat is applied very rapidly. We show this has a benefit on the size of the peptide fragments that we find in rat brains. We used our highly optimized and very fast peptide sequencing mass spectrometry platform to generate a large database of endogenous peptides extracted from hypothalamus, which comprises the biggest set of neuropeptides identified so far with more than 14,000 peptides found (Secher, Kelstrup et al., *Nature Communications*, 2016).

In another study, we revealed the molecular mechanisms explaining functional selectivity of different growth factors activating the same receptor on the plasma membrane. We deciphered the functional selectivity of growth factors differentially activating the EGF receptor in human cancer cells. To do this, we devised an Integrated Multi-layered Proteomics Approach (IMPA) to systematically examine how biased EGFR ligands affect signaling and cellular outputs (Figure 9). We investigated the cellular responses of the two EGFR ligands, epidermal growth factor (EGF) and transforming growth factor alpha (TGF-alpha), inducing EGFR degradation and recycling, respectively, and uncovered two fine-tuned regulatory molecular mechanisms. By integration of different layers of cellular information (EGFR interactome, ubiquitylome, phosphoproteome and proteome) we refined and prioritized protein candidates regulated in a ligand-dependent manner. In different human cancer cells, we demonstrated that EGF receptor degradation depends on EGF-induced phosphorylation of Rab7 on tyrosine 183 and that RCP (also known as RAB11FIP1) recruitment is necessary for EGFR recycling and sustained signaling upon TGF-alpha stimulation. By manipulating the expression level of RCP or phosphorylation of Rab7, EGF receptor trafficking can be rerouted thereby effectively changing the TGF-alpha specific cellular response to an EGF-like response and vice versa (Francavilla et al., *Nature Structural and Molecular Biology*, 2016).

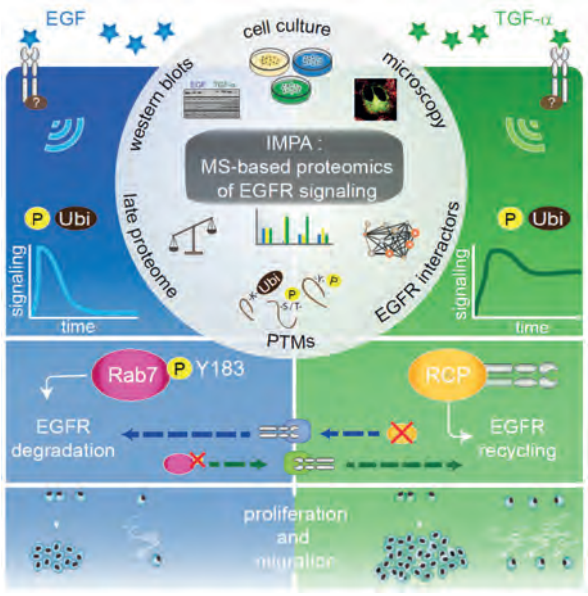


Figure 9 | Integrative Multi-layered Proteomics Approach (IMPA) developed to dissect the protein pathways responsible for ligand-dependent differential epidermal growth factor receptor signaling, trafficking and cellular outcome (Francavilla et al., *Nature Structural and Molecular Biology*, 2016).

MASS SPECTROMETRY PLATFORM

Professor
Michael L Nielsen, Platform Leader

Mass Spectrometry Specialist
Simone Schopper

The Mass Spectrometry Platform is headed by Professor Michael L Nielsen and operated by Mass Spectrometry Specialist Simone Schopper. The Platform was established in 2014 and currently provides technical support and maintenance for the research groups within the Proteomics Program in order to ensure that CPR retains state-of-the-art mass spectrometry technology. Additionally, the Platform provides analytical proteomics support for all CPR research groups and our collaborative stakeholders (Figure 10). As part of the analytical support, we collaborate closely with the Big Data Management Platform to ensure proper and efficient back-up possibilities for all data being acquired in the Mass Spectrometry Platform.

In 2016, Simone Schopper joined the Mass Spectrometry Platform. In her position as Mass Spectrometry Specialist, she provides analytical and technical support for all CPR research groups and oversees all maintenance tasks associated with the advanced mass spectrometric instrumentation available in the Platform (Figure 11).

Figure 10 | Overview of analytical services provided by the Mass Spectrometry Platform.

In early 2016, the Mass Spectrometry Platform upgraded all its ultra-high performance liquid chromatography (UHPLC) systems. To this end, we purchased 14 new UHPLC systems entailing intelligent maintenance and improved chromatographic performances. This new UHPLC 1200 system is designed with simplicity, reliability, and intuitive operation in mind, and includes optimized configurations for proteomic workflows. In combination with the arrival of Simone Schopper and our purchase in 2015 of an Orbitrap Fusion Lumos Tribrid mass spectrometer, the Platform now offers unprecedented analytical capacity to our collaborators at CPR as well as externally, offering state-of-the-art proteomics analysis at the highest international level.

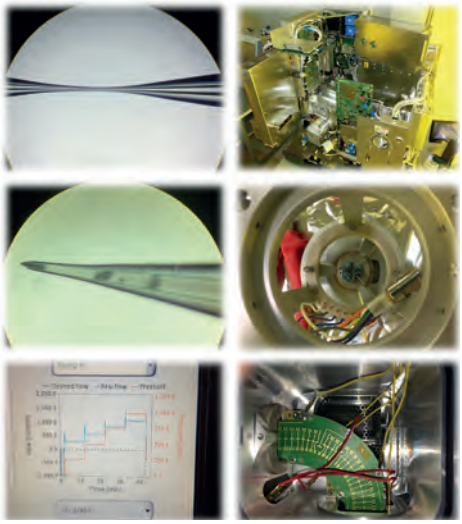


Figure 11 | The Mass Spectrometry Platform provides various technical support functions for the proteomics section, including column production and maintenance and repair of all liquid chromatography (LC) and mass spectrometry (MS) instrumentation.

DISEASE SYSTEMS BIOLOGY PROGRAM

Translational Disease Systems Biology
BRUNAK GROUP

Cellular Network Biology
JENSEN GROUP

Big Data Management Platform

FROM THE RESEARCH DIRECTOR

SØREN BRUNAK

"Big biomedical data have become a strategic priority worldwide, particularly in Denmark. The Disease Systems Biology Program at CPR is leading the development of innovative tools to analyze such data."

The Disease Systems Biology Program has a strong interest in human proteome variation and how this 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. We use essentially all data types of interest in biology and medicine: Traditional molecular level data, but also data from the healthcare sector, such as electronic patient records with text narratives, laboratory measurements, images, prescriptions, diagnoses, and procedures. 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 only focus on one disease in isolation but to conduct deep phenotyping of individual patients to get a clear picture of all disease comorbidities acquired over time.

Organization

The Program is led by Søren Brunak and consists of the Jensen group (Cellular Network Biology) and the Brunak group (Translational Disease Systems Biology). The Program is also responsible for the Big Data Management Platform, led by Lars Juhl Jensen, which provides supercomputer resources and advanced data storage schemes to research projects across CPR and its collaborative stakeholders. The Platform has taken advantage of the Danish National Life Science Supercomputer, Computerome, which is managed jointly by Technical University of Denmark (DTU), University of Copenhagen (UCPH) and Danish e-Infrastructure Cooperation (DeiC).

Technological advances

The Big Data Management Platform handles massive amounts of readouts from experiments as well as subsequent integrative analysis incorporating large external data sets. With more than 16,000 cores, Computerome is very powerful and is an infrastructure that is well suited for handling person-sensitive data in secure private cloud frameworks. The resources are used internally at CPR as well as in collaborations like those funded by the Innovative Medicine Initiative (IMI).

Research highlights

The STRING database of protein-protein interaction networks[1] is a major priority for our Program. In 2016 ,the Jensen group developed an app that provides easy access to protein associations from STRING through the Cytoscape network visualization and analysis tool[2]. With the new Cytoscape app we have greatly increased the utility of STRING in particular for researchers working with proteomics.

In the Brunak group, we have continued to develop our disease trajectory modeling framework that exploits clinical data from the entire Danish population. In two papers we have shown how time-ordered comorbidities can be analyzed over periods of more than 20 years (Beck et al., *Scientific Reports*, 2016; Beck et al., *Pacific Symposium on Biocomputing*, 2016). A major milestone was the contribution to a paper on how inflammatory diseases co-occur in millions of individuals from the Danish population and how those observations can be used to support interpretation of genomic genotyping studies of more than 85,000 patients and controls (Ellinghaus et al., *Nature Genetics*, 2016).

International standing and collaborations

Our Program has strong collaborative links to the other programs at CPR, supporting data analysis by offering supercomputer resources of the highest quality. In addition, we collaborate with CPR groups on specific research projects such as proteome-wide analysis of arginine monomethylation in collaboration with the Nielsen group (Larsen et al., *Science Signaling*, 2016).

Our Program collaborates with many external stakeholders in the clinical environment and elsewhere. A highlight of 2016 was securing funding for the project BigTempHealth (Innovation Fund Denmark) that addresses large-scale temporal analysis of health data from patients and is a collaboration between two Danish Regions (Capital Region of Denmark and Region Zealand), two hospitals (Rigshospitalet and Roskilde Hospital), two companies (Intomics A/S and Daintel A/S), and two universities (DTU and UCPH).

1. (http://string-db.org/)
2. (http://cytoscape.org/)

"We aim to combine molecular and clinical data in novel ways in order to provide deep phenotyping for use in precision medicine frameworks."

TRANSLATIONAL DISEASE SYSTEMS BIOLOGY

BRUNAK GROUP

Professor
Søren Brunak (Group Leader)

Affiliated Professor
Pope Moseley

Associate Professors
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Master's Students Mette K Pedersen, Muriel Heldring

Scientific Programmers
Piotr Chmura, Troels Siggaard, Mia L Værnda

Computer Specialist
Emil Karol Rydza

Chief Consultant
Peter Løngreen

EXTERNAL COLLABORATORS

Rudi GJ Westendorp and **Lene Juel Rasmussen** (Center for Healthy Aging, University of Copenhagen (UCPH), Denmark) disease progression modeling.

Torben Hansen and **Oluf Pedersen** (Novo Nordisk Foundation (NNF) Center for Basic Metabolic Research, UCPH, Denmark) diabetes patient stratification and metagenomics data integration.

Niels Tommerup (Wilhelm Johannsen Center, UCPH, Denmark) genome and chromosome structure analysis.

Peter Rossing (Steno Diabetes Center Copenhagen, Denmark) diabetes patient stratification, complication analysis, clinical biochemical data analysis, adverse outcome detection, polypharmacy.

Jens Lundgren (Rigshospitalet, Denmark) big biomedical data analysis on time-ordered comorbidities.

Anders Juul (Rigshospitalet, Denmark) systems biology in growth and reproduction.

Anders Perner (Rigshospitalet, Denmark) intensive care data analysis.

Pierre Baldi (University of California Irvine, US) deep learning models of clinical data.

Alfonso Valencia (Spanish National Cancer Research Centre (CNIO), Spain) bioinformatics and systems biology tools.

Chantal Mathieu (University of Leuven, Belgium) diabetes type 1 disease etiology.

Ewan Pearson (University of Dundee, UK), **Mark McCarthy** (Oxford University, UK), **Leif Groop** and **Paul Franks** (Lund University, Sweden) diabetes type 2 disease etiology and systems level analyses.

Ferran Sanz (University Pompeau Fabra, Spain) clinical bioinformatics.

Edith Heard (Institut Curie, France) X-inactivation related systems biology.

Simon Lovestone (Oxford University, UK) and **Johan van der Lei** (Erasmus University, The Netherlands) comorbidities and mental disorders.

INTERNAL COLLABORATORS

Matthias Mann, Atul Deshmukh (Mann group, Proteomics Program) establishing a joint effort on big data analysis of relevance for precision medicine based on clinical proteomics.

Guillermo Montoya, **Jesper V Olsen** (Montoya group, Protein Structure and Function Program and Olsen group, Proteomics Program) expression of human growth factors and characterization and discovery of their receptors.

Lars Juhl Jensen (Jensen Group, Disease Systems Biology Program) data integration, biomedical text mining and supercomputing.

2016

Challenges and research aims

One of the main challenges in the bioinformatics and systems biology areas is that biomedical data grows not only in volume, but also in heterogeneity. The scope of almost any data type expands at the molecular level, a trend one can exemplify by classical protein post-translational modifications, which increasingly become characterized in single sequences for a particular modification. At the same time, experimental techniques advance to produce data on many other types of modifications. This is just one example from the molecular level, but the same holds true at the clinical level, patients are increasingly screened with new assays, diagnosed using new medical ontologies and terminologies and in a country like Denmark, these data can on top be linked to a wealth of accessible, socio-economic information that have implications for health and disease. Via our collaborations with unique partners in healthcare and other large-scale data generating efforts the Brunak group is well positioned to meet this challenge. While clearly in need of continuous expansion, the computational infrastructure at CPR is suitable to support the current tasks.

Achievements

The major research aims of the Brunak group are related to solving the challenge of increasing data volume and heterogeneity. The challenge has a "chronic" aspect to it as experimental techniques continue to become more sophisticated while the world-wide political and societal ambition of treating individuals more effectively also provides strong support for expanding the big biomedical data field. The demographic changes in the Western societies add considerably to the push for revealing new disease mechanisms and inventing new data workflows and data processing algorithms, which can aid the development of more effective and possibly cheaper treatment interventions.

STRATEGIC GOALS

- To comprehensively develop new models for the analysis of disease progression and disease trajectories across the full spectrum of disease
- To construct novel patient stratification frameworks that can be used to deconvolute disease etiologies in broad patient groups and relate them to specific disease mechanisms in network biology frameworks
- To embark on the integration of data describing human genome/proteome variation with clinical phenotypic data and subsequently use these integrated models in the context of precision medicine

In 2016, the Brunak group worked across its research plan and covered several additional areas, both at the molecular and clinical data level as well as combinations of the two using integrative frameworks. We have continued to develop our disease trajectory modeling framework that exploits clinical data from the entire Danish population. In two papers, we have shown how time-ordered comorbidities can be analyzed over periods of more than 20 years. In one case we resolved the controversy in the literature on how diabetes impacts the survival of sepsis patients by subgrouping the patients according to their comorbidities (Beck et al., *Scientific Reports*, 2016). In another paper on sleep apnea and diabetes, we demonstrated that even if the order of two diagnoses in the population appears random (50:50), the actual order leads to widely different disease burdens (Beck et al., *Pacific Symposium on Biocomputing*, 2016).

Another 2016 highlight was the publication of an unsolicited paper in *Nature Reviews. Genetics* presenting a concerted view on the network biology concepts rewiring, robustness, and pleiotropy (Hu et al., *Nature Reviews. Genetics*, 2016). We use these concepts in network analysis of disease progression and the relation to genomic disease variants and environmental exposure. We also published high impact papers on human protein-protein interaction networks (Li et al., *Nature Methods*, 2016), integration of microbiome data in the study of insulin sensitivity (Pedersen et al., *Nature*, 2016), and pleiotropic analysis of co-occurring chronic inflammatory diseases (Ellinghaus et al., *Nature Genetics*, 2016).

Figure 12 I Søren Brunak inside the supercomputer container.

"The Cellular Network Biology group has developed tools that greatly simplify network-based analysis of omics data and diseases."

CELLULAR NETWORK BIOLOGY

JENSEN GROUP

Professor
Lars Juhl Jensen (Group Leader)

Postdoc
Nadezha Doncheva
David Lyon
Alberto Santos Delgado

PhD Students
Helen Victoria Cook
Oana Palasca
Xiaoyong Pan
Jan Refsgaard

Also featured in group picture:
Alexander Junge (Postdoc 2017)
Ufuk Kirik (Postdoc 2017)

INTERNAL COLLABORATORS

Ivo Hendricks (Nielsen group, Proteomics Program) global mass-spectrometry-based analysis of sumoylation.

Michael L Nielsen (Nielsen group, Proteomics Program) arginine methylation, sumoylation, and RNA-binding proteins.

Jesper V Olsen (Olsen group, Proteomics Program) statistical analysis of pull-down experiments.

Louise von Stechow (Olsen group, Proteomics Program) network-based analysis of phosphorylation sites.

EXTERNAL COLLABORATORS

Daniel Belstrøm (Section of Periodontology and Microbiology, University of Copenhagen (UCPH), Denmark) on metaproteomics analysis of saliva samples.

Thomas Frimurer (Novo Nordisk Foundation (NNF) Center for Basic Metabolic Research, UCPH, Denmark) on identification of novel small-molecule ligands for the US28 receptor.

Jan Gorodkin (Center for non-coding RNA in Technology and Health, UCPH, Denmark) integration of ncRNAs in protein networks for human and animal models; analysis of tissue expression data from mammalian model organisms.

Malene Maag Kristensen (Section of Systems Biology Research, UCPH, Denmark) statistical analysis of miRNAs and downstream responses to diet and exercise.

Alicia Lundby (Department of Biomedical Science, UCPH, Denmark) statistical analysis of pull-down experiments.

Peer Bork (European Molecular Biology Laboratory (EMBL), Germany) development of the eggNOG orthology resource, the STRING/STITCH network databases and the SIDER resource on adverse drug reactions.

Yesid Cuesto Astroz (Oswaldo Cruz Foundation, Belo Horizonte, Brazil) prediction of pathogen-host interactions.

Christian von Mering (University of Zurich, Switzerland) development of the eggNOG orthology resource and the STRING/STITCH network databases.

John "Scooter" Morris (University of California, San Francisco (UCSF), US) development of Cytoscape App for the STRING database.

Milana Frenkel-Morgenstern (Bar-Ilan University, Israel) text mining for cancer fusion proteins and their protein interactions.

Tudor Oprea (University of New Mexico, US) integrating and quantifying existing knowledge on drug targets.

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

Thomas Rattei (University of Vienna, Austria) development of the eggNOG orthology resource.

Quest for Orthologs Consortium benchmarking of orthology resources, including eggNOG.

2016

Challenges and research aims

High-throughput technologies revolutionize the way we study biological systems. 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 to develop better statistical methods for the analysis of mass spectrometry data. We develop community databases, web resources, and tools that aim to bring together high-throughput data-sets and, through the use of in-house text mining algorithms, information from the published literature.

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 text mining specifically. 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/tools 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 and post-translational modifications

Achievements

A major achievement this year was the development of an app that provides easy access to protein associations from STRING[1] through the Cytoscape network visualization and analysis tool[2]. The STRING database is maintained in close collaboration with research groups in Germany and Switzerland and is used by thousands of scientists around the world every day. Our publications about STRING have received more than 1900 new citations in 2015 and 2016 alone. With the new Cytoscape app, we have greatly increased the utility of STRING for researchers working with omics technologies, in particular proteomics, by enabling more advanced visualization and analyses of large protein networks (Figure 13). It also integrates with other databases from the group, including DISEASES, to allow users to easily retrieve a protein network for any disease of interest. The app was downloaded more than 5000 times in the first few months after its release. The work is described in the paper 'The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible' published in *Nucleic Acids Research* (Szklarczyk et al., *Nucleic Acids Research*, 2016).

We continue our efforts to develop text-mining tools that are efficient enough to be applied to the complete biomedical literature and easy enough to be used by non-experts. In 2016, we have participated in two international text-mining challenges, BioCreative and BioNLP, with excellent results in both (Pafilis et al., *Database*, 2016; Cook et al., *Proc BioNLP Workshop*, 2016). The text-mining software developed within the group was recently released as open source and is used by several other research groups, including collaborators on the National Institutes of Health program 'Illuminating the Druggable Genome'.

Last, but not least, we have continued our fruitful collaboration with the Proteomics Program on analysis of post-translational modifications, which has resulted in a joint publication in *Science Signaling* (Larsen et al., *Science Signaling*, 2016).

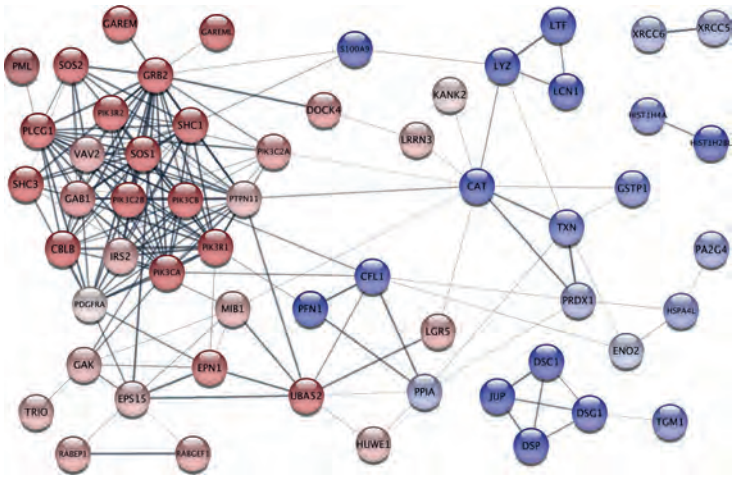


Figure 13 | STRING network visualization in Cytoscape. Using the Cytoscape STRING app, a network was retrieved for 78 proteins interacting with TrkA (tropomyosin-related kinase A) 10 min after stimulating neuroblastoma cells with NGF (nerve growth factor). The resulting network contains 182 functional associations between 57 of the proteins. Nodes are colored according to the protein abundance (log ratio) compared to the cells before NGF treatment. The confidence score of each interaction is mapped to the edge thickness and opacity (Szklarczyk et al., *Nucleic Acids Research*, 2016).

1. (http://string-db.org/)
2. (http://cytoscape.org/)

BIG DATA MANAGEMENT PLATFORM

Professor
Lars Juhl Jensen (Platform Leader)

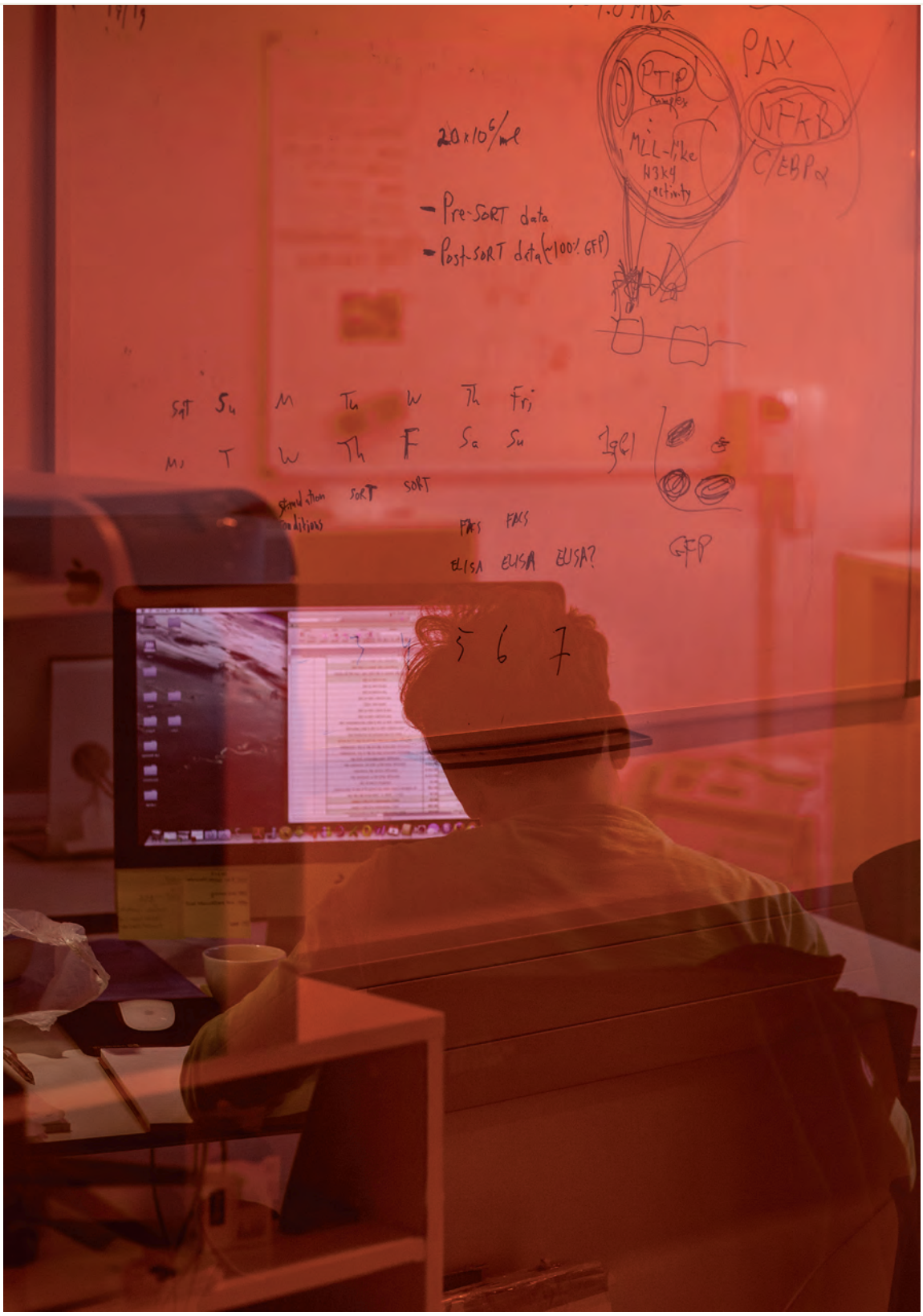
Computer Specialist
Rebeca Quinones

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 main users are the other Platforms, which need stable data management solutions, and not the majority of individual researchers at CPR.

In 2016, the Big Data Management Platform has focused on setting up a professional large-scale storage solution to capture instrument data and safely store, backup, and archive them. This system is now operational and manages the data from all other Platforms at CPR. The storage solution is physically collocated with the Danish Life-Science Supercomputer 'Computerome', which is managed jointly by Technical University of Denmark (DTU), University of Copenhagen (UCPH) and Danish e-Infrastructure Cooperation (DeiC). This supercomputer has enabled the Montoya group (Macromolecular Crystallography group, Protein Structure and Function Program) to perform highly demanding analyses of data of the 3D structures of proteins and complexes.

In the coming year, the Big Data Management Platform will focus on making it possible to use the Computerome facility for analysis of more types of data produced at CPR, in particular mass-spectrometry-based proteomics data. We will also explore the possibility to use the data storage solution to provide services for other Novo Nordisk Foundation centers.

Figure 14 | National Life Science Supercomputer: Computerome.


PAX
NFkB
C/EBPα
- 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

Mechanisms of DNA Repair and DNA Replication
DUXIN GROUP

Chromosome Stability and Dynamics
LUKAS GROUP

Ubiquitin Signaling
MAILAND GROUP

Mitotic Mechanisms and Regulation
NILSSON GROUP

Protein Imaging Platform

FROM THE RESEARCH DIRECTOR

JIRI LUKAS

"All groups made significant, in some cases paradigm-shifting, contributions towards understanding how established and novel protein pathways keep our genomes free of disease-predisposing alterations."

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, aging and hematopoietic and immune deficiencies.

Organizational development

2016 was marked by the recruitment of junior group leader Julien Duxin ('Mechanisms of DNA Repair and DNA Replication' group). Julien comes from a postdoc position in Johannes Walter's lab at Harvard Medical School (US) and we are extremely happy that he chose our Program to develop his independent research career. 2016 also saw the successful quinquennial review of group leader Jakob Nilsson who obtained his professorship with a very strong scientific program for the next 5 years focused on protein phosphatases and their role in cell cycle and genome integrity regulation.

Technological advances

The Program is continuously developing our technological capabilities, not least thanks to the close link to the Protein Imaging Platform, headed by Claudia Lukas. Scientists from all the groups in the Program interact closely with the Protein Imaging Platform as users and as active contributors, to improve and develop the analytical possibilities of our microscopes and the power of advanced image analysis. The technological advantage brought about by the interaction with the Protein Imaging Platform is exemplified by the systematic genetic screens performed in the Lukas group that provides a rich data resource describing the consequences of failed chromosome segregation to the genomic fitness in subsequent cell generations.

The arrival in 2016 of Julien Duxin and his group brings unique expertise in cell-free biochemistry, which is a huge boost to our analytical portfolio, not only for the Program but all of CPR.

Research highlights

IIn 2016, all groups made important discoveries, published without exception in top international journals, and in many cases achieved through in-house synergy. An outstanding example from the Mailand group is the identification of the ETAA1 protein, a novel activator of one of the major protein phosphorylation signaling cascades activated by DNA replication stress (Haahr et al., *Nature Cell Biology*, 2016). There is a huge potential in this discovery to gain a deeper understanding of some of the main cell-fate decision during cancer development. The Mailand and Daniel groups joined forces to characterize SCAI, another novel genome caretaker involved in chromatin-context specific DNA repair in somatic cells and during meiotic recombination in germ cells (Hansen et al., *Nature Cell Biology*, 2016). The Nilsson group contributed to the joint scientific endeavor by the equally paradigm-setting discovery of a consensus binding motif for a major mammalian protein phosphatase, PP2A-B56 (Hertz et al., *Molecular Cell*, 2016). This work benefited from interaction across CPR programs (Olsen and Montoya groups) and has an immense potential to place CPR at the cutting edge of protein phosphatase research. Finally, the Lukas group contributed to the impressive scientific output of the program also by a paradigm shifting concept of the role of DNA damage mediator 53BP1, which turns out to be not a general inhibitor of homology-directed repair of DNA double strand breaks, but a fidelity maker of this essential component of genome integrity maintenance (Ochs et al., *Nature Structural and Molecular Biology*, 2016).

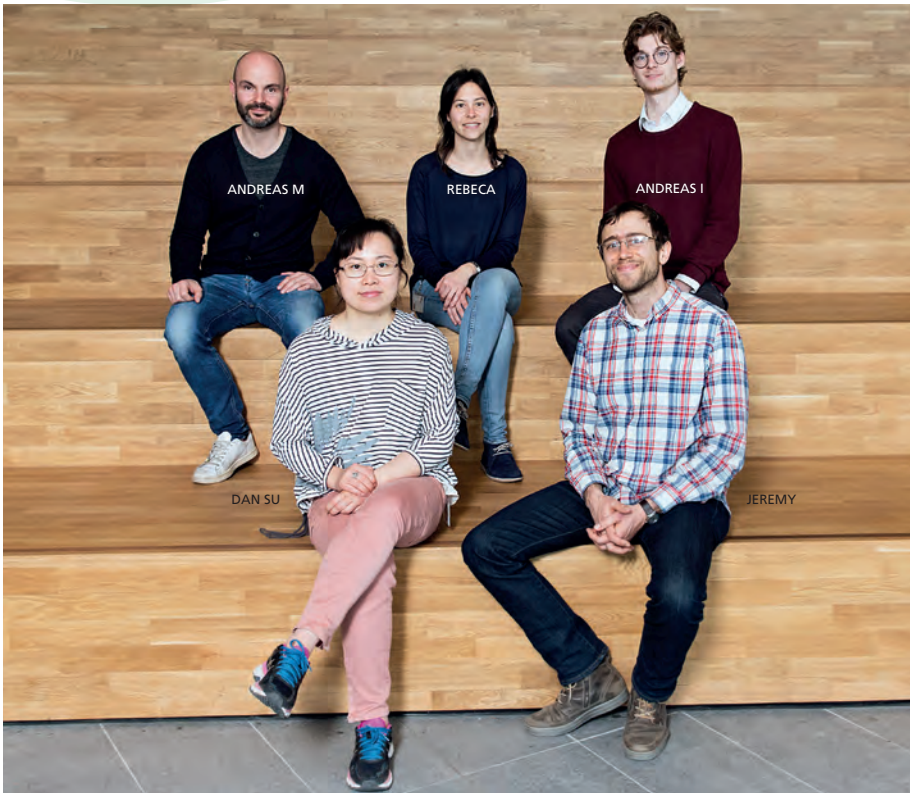
International standing and collaborations

The success of our Program relies strongly on synergistic scientific interactions with other Programs and active contributions to CPR's technological Platform concept as clearly demonstrated by the joint scientific endeavors described in the previous section. Equally important, our Program has strong interactions with research groups in Europe and the US, but importantly also with influential scientists in the Copenhagen area, including other Novo Nordisk Foundation Centers, Center for Chromosome Stability, and Biotech Research and Innovation Center (BRIC).

"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
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Linda Starnes

PhD Student
Laura Pikkupeura

Technicians
Martina Kubec Højfeldt
Rebeca Soria Romero

Laboratory Assistant
Andreas Ingham

INTERNAL COLLABORATORS

Michael L Nielsen (Nielsen group, Proteomics Program) identifying functional proteins at DNA breaks with quantitative proteomics.

Jiri Lukas (Lukas group, Protein Signaling Program) identifying functional proteins at DNA breaks with quantitative proteomics.

Niels Mailand (Mailand group, Protein Signaling Program) elucidating the biological roles of DNA damage response factors.

Guillermo Montoya (Montoya group, Protein Structure and Function Program) elucidating the function of the PTIP/PA1 subcomplex in transcription.

EXTERNAL COLLABORATORS

Andres Contreras-Lopez (Center for Chromosome Stability, University of Copenhagen (UCPH), Denmark) the role of replicative stress during immune cell development and aging.

Joan Yuan (Lund University, Sweden) the roles of DNA damage during immune cell development and aging.

Andre Nussenzweig (National Cancer Institute/ National Institutes of Health (NCI/NIH), US) elucidating DNA repair pathways disrupted in cancer.

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

Francesca Cole (MD Anderson/University of Texas, US) elucidating the roles of DNA damage response factors during meiosis.

2016

Challenges and research aims

We are interested in how DNA damage and genomic instability impact normal development and aging. While the field understands that certain cell-type specific DNA damage of our genome generates antibody diversity and is greatly beneficial, the negative impacts that various other DNA damage stresses have on our immune system remain incompletely understood. Unresolved DNA damage in blood cells not only leads to immunodeficiency but also cancer and other age-related pathologies. We believe cell signaling incurred in response to DNA damage has important and still undefined consequences for the differentiation and regeneration of blood cells, particularly during aging. This challenge is the main priority of our group. We are working on the hypothesis that DNA damage, including lesions incurred during DNA replication, impacts the clonal behavior of self-renewing blood cells and their differentiation potential. Our group is well placed to tackle this challenge with our expertise in DNA damage, mouse genetic models and immune cell biology, and we hope continued work in this area inspires new therapeutic strategies to modulate DNA damage signaling for improving immune cell function.

Achievements

In collaboration with the Mailand group, we discovered a novel 53BP1-interacting factor in the DNA damage response called SCAI and characterized its physiological function in mice. We found that SCAI exhibits an atypical dual recruitment pattern to DNA breaks and, using targeted gene inactivation in mice, discovered that SCAI promotes DNA double-strand break (DSB) repair in at least two different chromosomal contexts: the first as a specific mediator of ATM/53BP1-dependent heterochromatic DSB repair and the second as a mediator of homologous recombination (HR) during meiotic progression in germ cells. The findings uncover SCAI as an important and specialized component of both NHEJ- and HR-mediated pathways that potentiates DSB repair efficiency in specific chromatin contexts (Figure 15). This discovery is clearly in line with one of our group's main strategic goals of elucidating the physiological roles of DNA damage response factors. This work was published in *Nature Cell Biology* (Hansen et al., *Nature Cell Biology,* 2016). Additional collaborative work by the group in 2016 was published in *Nature*, *Immunity*, and *Science Signaling*, spanning the fields of DNA repair, lymphocyte development, and post-translational modifications (Chaudhuri et al., *Nature*, 2016; Kristiansen et al., *Immunity*, 2016; Larsen et al., *Science Signaling*, 2016).

STRATEGIC GOALS

- To elucidate the physiological roles of DNA damage response factors during lymphocyte development and the maintenance of genome stability
- To develop a biochemical methodology coupling chromatin immunoprecipitation with quantitative proteomics for identifying novel proteins associated with DNA damage

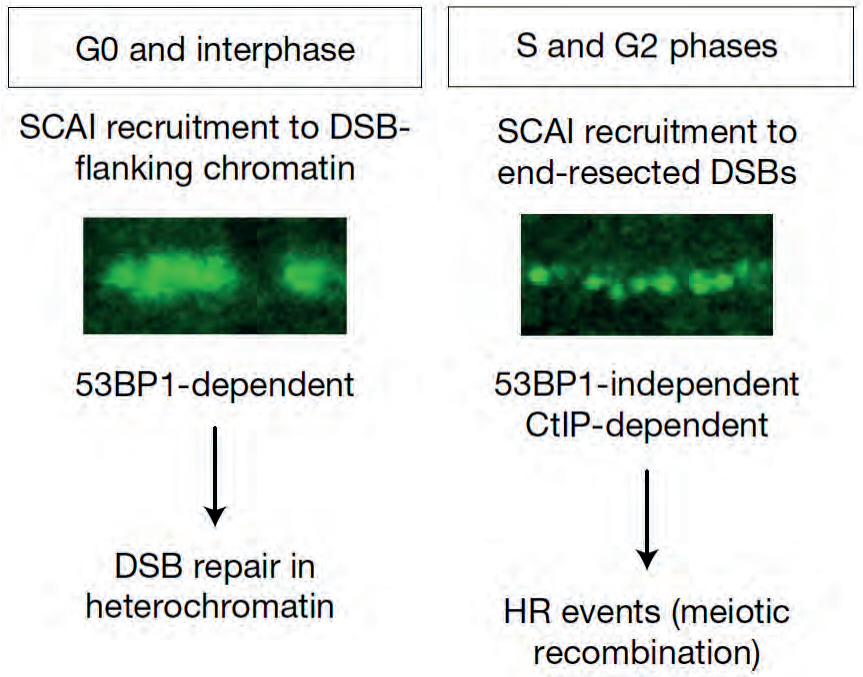


Figure 15 | Model for how the SCAI protein functions in DNA double-strand break (DSB) repair. SCAI is recruited to DSB-proximal chromatin throughout interphase through direct interaction with 53BP1, promoting 53BP1- and ATM-mediated repair of heterochromatic DSBs. During the S and G2 phases of the cell cycle, SCAI also accumulates at CtIP-resected ssDNA regions in a 53BP1-independent manner. From this locale, SCAI supports a subset of homologous recombination events and its deficiency is associated with defects in meiotic recombination and germ cell development (Hansen et al., *Nature Cell Biology*, 2016).

"We use protein extracts derived from Xenopus laevis eggs to recapitulate and understand essential processes of genome duplication and maintenance."

MECHANISMS OF DNA REPAIR AND DNA REPLICATION

DUXIN GROUP

Associate Professor
Julien Duxin (Group Leader)

Postdocs
Irene Gallina
Nicolai Larsen

Research Assistant
Matilde Hyldig

Laboratory Assistant
Andreas Ingham

Also featured in group picture:
Lisa Schubert (PhD Student in Mailand group)

INTERNAL COLLABORATORS

Niels Mailand (Mailand Group, Protein Signaling Program) exploration of DNA damage signaling in Xenopus egg extracts.

Michael L Nielsen (Nielsen Group, Proteomics Program) identification of novel repair factors in Xenopus egg extracts by mass spectrometry.

2016

Challenges and research aims

Our cells are under continuous assault from reactive compounds that damage their DNA. To counter the accumulation of DNA lesions, cells have evolved specialized repair pathways that are remarkably conserved throughout evolution. We are particularly interested in delineating repair pathways that are coupled to DNA replication as the process of copying the genome often triggers DNA repair. Conversely, failure to efficiently replicate the genome undergoing genotoxic stress is linked to a variety of cancer and accelerated aging syndromes. By delineating these repair mechanisms, we aim to identify the causes underlying these genetic disorders and provide important molecular clues that will lead to better treatments in the clinic.

In our group, we use protein extracts derived from eggs of the African clawed frog, Xenopus laevis. These protein extracts have the extraordinary capacity to reiterate in a test tube the fundamental processes of genome duplication and maintenance. The use of these extracts recently led to the discovery of a novel repair pathway that specifically removes DNA-protein cross-links during S phase (Duxin et al., *Cell*, 2014[1]). DNA-protein cross-links, also known as DPCs, are toxic DNA lesions induced by a variety of endogenous or exogenous cross-linking agents and are suspected to cause cancer and aging. When a replication fork encounters a DPC, the replisome stalls due to the collision of the replicative helicase with the protein adduct (Figure 16, i). The DPC is then proteolytically degraded into a peptide adduct (Figure 16, ii) that is amenable for replication fork bypass (Figure 16, iii). This repair process illustrates the incredible orchestration of events that must occur at the replication fork in order to specifically target toxic protein adducts for degradation while maintaining a functional replisome. In the Duxin group, we are interested in understanding how these different events are coordinated and identify the key players that participate in each of the steps so that we can understand this reaction in great detail.

STRATEGIC GOALS

- To identify and characterize the key proteins that participate in the repair of toxic DNA lesions known as DNA-protein cross-links
- To comprehensively understand how the wide diversity of proteins cross-linked in the genome are specifically targeted for degradation during DNA replication

Achievements

The 'Mechanisms of DNA replication and DNA repair' group was established in August 2016. The first 6 months were focused on establishing the Xenopus egg extract system at CPR and developing new projects and collaborations. We are now working full speed, producing our own egg extracts, which we are using to address elemental question related to replication-coupled repair mechanism. A major highlight of 2016 was that group leader Julien Duxin attracted a prestigious ERC Starting Grant from the European Research Council (ERC).

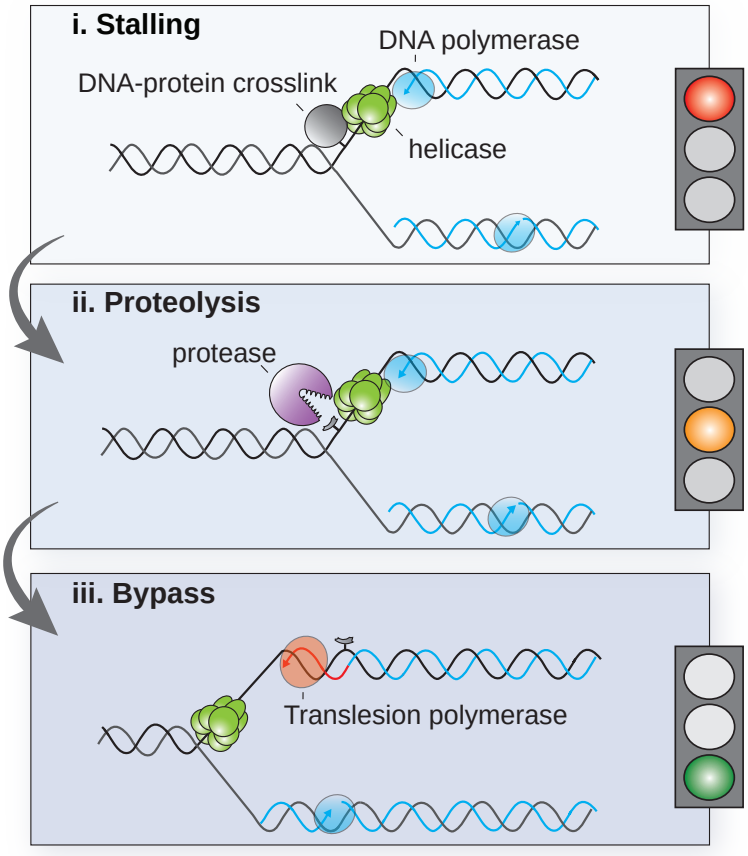


Figure 16 | Mechanism of DNA-protein cross-link repair in S phase (graphical abstract adapted from Duxin et al., *Cell*, vol 159, p346-57, 2014). See text for more details.

1. Repair of a DNA-protein crosslink by replication-coupled proteolysis. Duxin, JP, Dewar, JM, Yardimci, H & Walter, JC. *Cell* vol 159, pp. 346-57 (2014)..

"By combining genetic silencing, quantitative imaging and biochemical analysis of protein pathways activated by endogenous sources of DNA damage, we aim to understand 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
Kai Neelsen
Kumar Somyajit
Luis Toledo
Ronni Sølvhøj Pedersen
Julian Spies

PhD Students
Fena Ochs
Hana Sedlackova

Technicians
Maj-Britt Druedahl Rask
Merete Grøfte

Laboratory Assistant
Philip Becher Jørgensen

INTERNAL COLLABORATORS

Jesper V Olsen (Olsen group, Proteomics Program) the role of ATM and ATR phosphorylation during DNA replication catastrophe.

Rajat Gupta and **Chunaram Choudhary** (Choudhary group, Proteomics Program) the dynamics of proteins governing DNA replication and functional characterization of new genome caretaker proteins.

Bhargav Saligram Prebhkar and **Guillermo Montoya** (Montoya group, Protein Structure and Function Program) the structure and function of replicative helicase at the end of the DNA replication cycle.

EXTERNAL COLLABORATORS

Center for Integrated Microscopy (CFIM, University of Copenhagen (UCPH), Denmark) super-resolution microscopy.

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) the role of mitotic perturbations on genome integrity in the subsequent cell generations.

Andre Nussenzweig (National Cancer Institute/National Institutes of Health (NCI/NIH), US) protein signaling pathways subverted in cancer.

Neil Henderson (University of Edinburgh, UK) the role of DNA damage signaling in tissue differentiation.

Lothar Shermelleh (University of Oxford, UK) structured illumination microscopy as a tool to dissect protein complexes that shield DNA double-strand breaks against excessive nucleolytic erosion.

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

2016

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 where 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 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 questions, thanks to our longstanding innovative approaches to visualize cellular responses to genotoxic stress in their physiological environment, i.e. the nucleus of a living cell.

Achievements

2016 has been marked by the completion of two ambitious and long-term projects. First, in work performed by Fena Ochs in close collaboration with Kumar Somyajit and supervised by Claudia Lukas, we have discovered how human cells navigate repair proteins to fix DNA double-strand breaks without errors and thereby keep the genome disease-free (Ochs et al., *Nature Structural and Molecular Biology*, 2016). We found that 53BP1, an established chromatin-associated genome caretaker, is the critical factor in this process thanks to its ability to limit the extent of DNA-end resection. This suppresses a toxic form of repair called single-strand annealing and ensures that DNA double-strand breaks are repaired via the error-free mechanism gene conversion (Figure 17).

STRATEGIC GOALS

- To identify rate-limiting proteins and protein modifications that determine the dynamics of DNA replication and fidelity of DNA repair
- To elucidate the crosstalk between mitotic errors and replication stress in generating and propagating endogenous DNA damage across cell populations

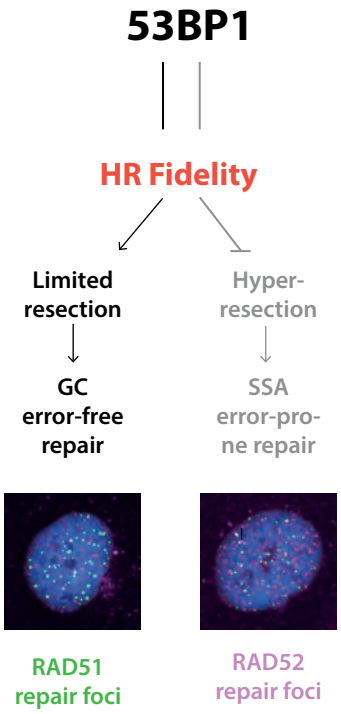


Figure 17 | Graphical model showing the impact of a 53BP1-mediated shield against excessive DNA-end resection on repair fidelity of DNA double strand breaks. The mechanism was published in *Nature Structural and Molecular Biology*, (Ochs et al., 2016).

This work illuminates how human cells repair serious assaults to their genomes without mistakes and what the molecular limits of this high-fidelity form of DNA repair are, but our work also has potential medical implications, for instance by suggesting that survival of some forms of drug-resistant tumors may heavily rely on the error-prone repair mechanisms. This opens up a possibility to search for drugs that would target such repair pathways and thereby re-sensitize advanced cancers to treatment.

Second, thanks to work by Ronni Sølvhøj Pedersen in close collaboration with Gopal Karemore (Protein Imaging Platform), we have completed a study utilizing a large image-based genetic screen to elucidate how primary errors during chromosome segregation in mitosis destabilize the genome of ensuing cell generations (Pedersen et al., *Nature Communications*, 2016). This work provides a rich and well-curated data resource to assess the magnitude and dynamics of DNA damage caused by a broad range of mitotic errors and identifies replication stress as a mediator of DNA breakage in a subset of such events.

Finally, our laboratory together with the Protein Imaging Platform and in close collaboration with colleagues at European Molecular Biology Laboratory (EMBL) have much advanced a large screening project aiming at identifying and characterizing novel genome caretaking proteins thus far conceded in the uncharacterized protein-coding fraction of the human genome. Completion of this project, including release of the dataset to the scientific community, is amongst the top ambitions of the Lukas group for the coming year.

"We combine systems-wide and focused approaches within protein science to explore and dissect the cellular signaling processes that protect the genome from the harmful consequences of DNA damage and replication stress."

UBIQUITIN SIGNALING

MAILAND GROUP

Professor
Niels Mailand (Group Leader)

Associate Professor
Simon Bekker-Jensen

Postdocs
Petra Schwertman
Claire Guerillon
Teresa Ho
Dimitris Typas

PhD Students
Saskia Hoffmann
Peter Haahr
Divya Achuthankutty
Nikoline Borgermann
Rebecca Kring Hansen
Stine Smedegaard
Anita Ripplinger
Maxim Tollenaere
Lisa Schubert

Master's Student
Jonas Damgaard Elsborg

Laboratory Assistant
Julie Nielsen

Also featured in group picture:
Robert Francis Shearer (Postdoc ,2017)
Kathrine Weischenfeldt (Technician, 2017)

INTERNAL COLLABORATORS

Michael L Nielsen (Nielsen group, Proteomics Program) proteomic analysis of signaling processes in the DNA damage response.

Chunaram Choudhary (Choudhary group, Proteomics Program) proteomic analysis of new factors in genome stability maintenance pathways.

Julien Duxin (Duxin group, Protein Signaling Program) exploration of DNA damage signaling in Xenopus egg extracts.

Guillermo Montoya (Montoya group, Protein Structure and Function Program) biophysical and structural analysis of components of the DNA damage response.

EXTERNAL COLLABORATORS

Andres Lopez-Contreras (Center for Chromosome Stability, University of Copenhagen (UCPH), Denmark) generation and characterization of knockout mouse models for new factors in replication stress responses.

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

Andre Nussenzweig (NCI/NIH, US) ubiquitin-dependent signaling in DNA double-strand break repair using mouse models.

Titia Sixma (Netherlands Cancer Institute, the Netherlands) biochemical and structural characterization of factors in the ubiquitin network.

Matthias Mann (Max Planck Institute of Biochemistry, Munich, Germany) proteomic mapping of proteins acting in the context of DNA lesions.

2016

Challenges and research aims

We aim to understand how the cellular responses that protect the integrity of genetic material following genotoxic insults such as DNA damage and replication stress are orchestrated and regulated in space and time. Correct functioning of these processes is of fundamental importance to avoid multiple pathologies that can arise due to genetic changes. While it is clear that protective cellular responses to genotoxic insults are largely driven by coordinated post-translational modifications of the numerous proteins involved, we are still far from a complete picture of the scope, dynamics and functions of these crucial yet complex processes. Our group is excellently positioned to address this challenge due to our long-standing expertise in dissection of cellular signaling processes by means of cell biology-, biochemistry- and microscopy-driven approaches. Through collaboration with leading in-house and external experts in proteomics, model organisms and structural biology, we employ innovative approaches to understand the signaling responses promoting genome stability maintenance on both a systems-wide and focused level.

STRATEGIC GOALS

- To discover and functionally characterize key regulators of genome stability maintenance pathways in mammalian cells
- To understand how signaling by ubiquitin and ubiquitin-like modifier proteins promote cellular responses to DNA damage and replication stress

Achievements

In collaboration with Matthias Mann's lab in Munich, we recently developed a powerful method, termed CHROMASS, for systematic identification of the cellular proteins undergoing enrichment at DNA lesions (Räschle et al., *Science*, 2015[1]). In 2016, in line with the strategic goals of the group, we continued to utilize this pioneering approach to probe responses to different types of DNA damage on a global scale and to functionally characterize a range of hitherto unrecognized genome stability maintenance factors that we uncovered with this methodology. Among these, we demonstrated that the uncharacterized protein ETAA1 has an important role in protecting genome stability and cell survival following replication stress by serving as an activator of the ATR kinase (Figure 18). The paper (Haahr et al., *Nature Cell Biology*, 2016) was highlighted in a News and Views article in the same issue). This insight may provide new opportunities for promising current therapeutic strategies targeting ATR and replication stress in cancer cells.

We also discovered that the protein SCAI promotes DNA double-strand break repair in different chromosomal contexts (Hansen et al., *Nature Cell Biology*, 2016). Furthermore, we uncovered an important role of the ubiquitin ligase TRAIP in promoting signaling responses to replication stress (Hoffmann et al., *Journal of Cell Biology*, 2016). In addition, we published a comprehensive review on regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers, a research area of major interest for the lab (Schwertman et al., *Nature Reviews. Molecular Cell Biology*, 2016).

A current focus of the group is to gain in-depth understanding of the cellular roles of above-mentioned and other new DNA damage response components that we have identified in recent years. Through ongoing collaborations with in-house, local and external partners, we are studying the mechanisms and functions of these proteins by approaches ranging from structural analysis of individual protein domains to generation of mouse knockout models to determine their physiological roles in health and disease.

Another key priority for the lab is to develop extensions of CHROMASS and related methods for enriching proteins at genotoxic stress sites as a means to unravel the landscape of protein post-translational modifications such as ubiquitylation that occur locally in the vicinity of sites of DNA damage and replication stress. It is our conviction that successful implementation of such methodology would enable us to illuminate the functions, dynamics and complexity of DNA damage signaling mechanisms in unprecedented detail.

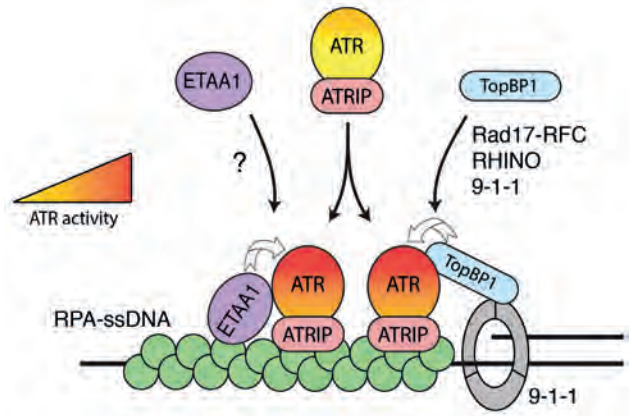


Figure 18 | A model for how ETAA1, a new and important factor in the DNA damage response identified by our lab, promotes activation of the ATR kinase, a master organizer of cellular responses to replication stress (Haahr et al., *Nature Cell Biology*, 2016).

1. Proteomics reveals dynamic assembly of repair complexes during bypass of DNA cross-links. Räschle, M, Smeenk, G, Hansen, RK, Temu, T, Oka, Y, Hein, MY, Nagaraj, N, Long, DT, Walter, JC, Hofmann, K, Storchova, Z, Cox, J, Bekker-Jensen, S, Mailand, N & Mann, M. *Science*, vol 348, p. 1253671 (2015).

"We aim to understand how signal transduction pathways are regulated by phosphorylation to achieve precise temporal control of cell division."

MITOTIC MECHANISMS AND REGULATION

NILSSON GROUP

Associate Professor
Jakob Nilsson (Group Leader)

Associate Professors
Thomas Kruse

Postdocs
Gang Zhang
Marie Sofie Yoo Larsen

PhD Students
Emil Peter Thrane Hertz
Jamin Hein

Research Assistant
Dimitriya Hristoforova Garvanska

Laboratory Assistant
Amanda Gammelby Qvesel

INTERNAL COLLABORATORS

Chunaram Choudhary (Choudhary group, Proteomics Program) regulation of anaphase promoting complex (APC/C).

Jesper V Olsen (Olsen group, Proteomics Program) mechanism of protein phosphatases.

Michael L Nielsen (Nielsen group, Proteomics Program) novel methods for identifying protein interactions in the mitotic checkpoint.

Guillermo Montoya (Montoya group, Protein Structure and Function Program) structural and biophysical characterization of protein phosphatases and checkpoint proteins.

EXTERNAL COLLABORATORS

Michael Lisby (Institute of Biology, University of Copenhagen (UCPH), Denmark) analysis of BRCA2 biology.

Marie Kveiborg (Biotech Research and Innovation Center (BRIC), UCPH, Denmark) ADAM17 regulation by phosphorylation.

Kristian Strømgaard (Department of Drug Design and Pharmacology, UCPH, Denmark) peptide inhibitors against PP2A-B56.

Norman Davey (University College Dublin, Ireland). Analysis of motifs for protein phosphatases.

Stephan Becker (Philipps University Marburg, Germany) role of protein phosphatases in filovirus biology.

Ylva Ivarsson (Uppsala University, Sweden) protein tilling (targeting-induced local lesions in genomes) of protein phosphatases.

2016

Challenges and research aims

Decades of research have shown that reversible phosphorylation of proteins, executed by kinases and phosphatases, constitutes a major form of signaling and an essential mechanism of regulation in all living organisms. Despite their importance very little is known about protein phosphatase regulation of signaling and phosphatases are often mistakenly considered unspecific. One of the major reasons for this is our limited understanding of phosphatase substrate recognition. In our view, deciphering the mechanisms of phosphatase substrate recognition would provide the means to significantly advance our understanding of phosphorylation mediated signaling in eukaryotes. CPR provides a unique scientific environment for achieving this goal through integration of its technology Platforms in a collaborative effort placing us in a unique and strong position to tackle protein phosphatase biology.

STRATEGIC GOALS

- To understand how protein phosphatases achieve substrate specificity and how this facilitates the proper segregation of genetic material during mitosis
- To understand how major cell cycle transitions are controlled through regulation of the APC/C ubiquitin ligase

Achievements

We have defined a consensus binding motif for the major cellular protein phosphatase, PP2A-B56, allowing us to uncover how this phosphatase regulates cellular signaling in numerous disease relevant pathways (Figure 19; Hertz et al., *Molecular Cell*, 2016). The paper was highlighted by *Nature Reviews. Molecular Cell Biology* and generated three invited institute research seminars and three oral presentations at major conferences. This is the first example of how specificity is achieved by PP2A and research into its mechanisms of function has been an important strategic aim of the group. It provides a solid foundation for dissecting PP2A-B56 function in relation to human diseases in the future and for developing specific inhibitors against this complex.

Furthermore, we have defined two novel pathways regulating Cdc20, the co-activator of the anaphase promoting complex (APC/C). It is important to understand regulation of the APC/C because inhibitors against this complex are being tested in clinical trials and the fundamental insight we provide is important for interpreting the outcomes of these trials. Firstly, we found a G2 pathway regulating cell growth through phosphorylation of Cdc20 that helped us reveal a coupling between different cell cycle states (Hein and Nilsson, *Nature Communications*, 2016). As cancer cells display uncontrolled cell growth, we have uncovered that this pathway could potentially serve as an Achilles heel for targeting cancer cells. Secondly, we provided insight into how Cdc20 is inhibited during cell division by defining two distinct pools of the checkpoint protein BubR1 on kinetochores (Zhang et al., *Nature Communications*, 2016). We found that only one of the BubR1 pools is able to inhibit Cdc20 providing important insight into the kinetochore in this process.

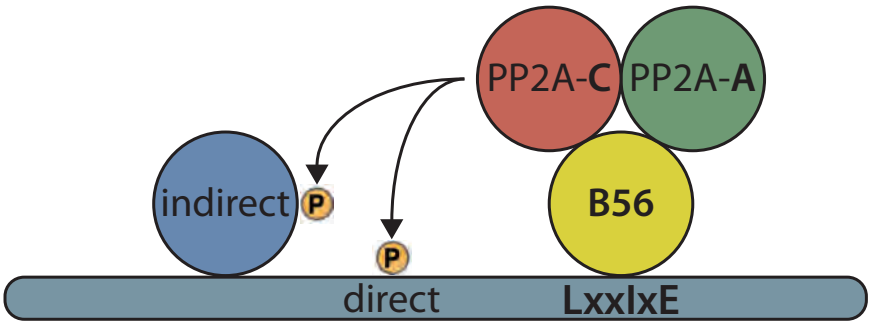


Figure 19 | A molecular description of protein phosphatase 2A (PP2A) binding specificity in eukaryotes. The PP2A-B56 holoenzyme binds to short linear LxxIxE motifs on interacting proteins. The LxxIxE consensus sequence is recognized by a conserved binding pocket on B56 regulatory subunits. The composition of the motif modulates the affinity for B56, which in turn determines the phosphorylation status of associated substrates. With this study, we provide the molecular details for binding specificity of PP2A, the most abundant source of phosphatase activity in the cell, which has broad implications for understanding signaling in eukaryotes (Hertz et al., *Molecular Cell*, 2016).

PROTEIN IMAGING PLATFORM

Professor
Claudia Lukas (Platform Leader)

Microscopy Specialist
Jutta Bulkescher

Image Analysis Specialist
Gopal Karemore

Also featured in picture:
Kai Neelsen (Postdoc in Lukas group and part time associated with Platform from 2017)

The purpose of the Protein Imaging Platform is to provide support to all research groups at CPR that use microscopy as a tool to investigate protein function. We aim to provide state-of-the-art tools for image-based, quantitative cell biology and at the same time train the new generation of scientists in essential light microscopy and enable them to make full use of the opportunities afforded by bioimaging.

The majority of students and postdocs that we support with microscopy and image analysis come from research groups in the Protein Signaling Program. Here, studies on protein localization in the cellular space using confocal imaging, live-cell imaging and protein recruitment to laser-induced DNA damage are very popular imaging applications. We also have many users seeking assistance for high-content microscopy and quantitative microscopy of multi-parametric, cell-cycle resolved phenotypes. In addition, we support a number of scientists from the Proteomics Program that apply microscope-based tools to explore the function of proteins identified by proteomic methods, including functional studies involving siRNA-based perturbations.

We work closely with the Big Data Management Platform to safeguard microscopy data storage and obtain access to powerful computer processing of large datasets at the 'Computerome' supercomputer. We also have strong links to the Danish Stem Cell

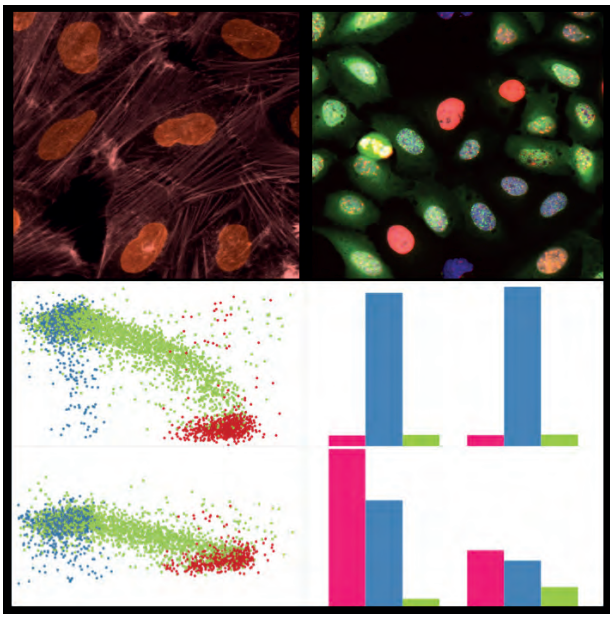


Figure 19 | The illustration shows an example of the imaging and data analysis that the Protein Imaging Platform provides for its users.

Center (DanStem, University of Copenhagen (UCPH)) since our platform experts Jutta Bulkescher and Gopal Karemore are shared staff with DanStem, just like we share Danstem's instrumentation for Flow Cytometry and Cell Sorting.

The Protein Imaging Platform has great emphasis on training of our daily users as well as teaching at courses and workshops. We organize regular training sessions for basic and advanced imaging and invite external speakers for workshops and seminars. In 2016 we organized practical microscopy and image analysis exercises during the CPR Summer School course for pre-graduate students (UCPH) and were invited to lecture and teach microscopy on a practical course for high-throughput microscopy at European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany).

In order for the platform support to stay cutting-edge we are continually developing novel assays in image acquisition and analysis. In addition, our staff participates in workshops, courses and conferences to be inspired and learn new methods in microscopy. In order to facilitate access to emerging technologies, such as super-resolution microscopy and to develop novel protocols for advanced image analysis, we maintain a close connection to other imaging facilities and actively network in the local and international bioimaging community, especially the Advanced Light Microscopy Facility (ALMF) at EMBL (Germany), the Advanced Bioimaging Unit Micron at Oxford University (UK) and imaging scientists at Institute of Molecular Biology (IMBA, Austria).

PROTEIN STRUCTURE AND FUNCTION PROGRAM

Macromolecular Crystallography

MONTOYA GROUP

Protein Production and Characterization Platform

FROM THE RESEARCH DIRECTOR

GUILLERMO MONTOYA

"We have implemented high-resolution cryo-EM procedures in the analysis of protein complexes relevant to cell cycle progression and DNA repair involved in key cellular processes"

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

The Protein Structure and Function Program is composed by the Montoya group (Macromolecular Crystallography) and the Protein Production and Characterization Platform. The Platform is organized in three teams involved in protein production and characterization. In 2016, University of Copenhagen (UCPH) was granted 60 million DKK (7.8 million EUR) by the Novo Nordisk Foundation (NNF) to establish a cryo-electron microscope (cryo-EM) facility. The cryo-EM is placed at the Core Facility for Integrated Microscopy (CFIM) at the Faculty of Health and Medical Sciences and will be anchored at CPR. Thanks to this generous grant, CPR will recruit a junior group leader within the field of macromolecular complexes and cryo-EM to join the Protein Structure and Function Program.

Technological advances

We have continued the implementation of high-resolution cryo-EM procedures for the analysis of protein complexes relevant to cell cycle progression and DNA repair involved in key cellular processes. The implementation of cryo-EM was initiated in earlier years and will culminate in 2017 with the arrival of a cryo-EM junior group and the cryo-EM facility at CFIM being fully operational. In 2016, the Protein Production and Characterization Platform has updated its protocols moving from a high throughput production to a project-specific scheme and has also established high throughput calorimetry, which allows the measurements of hundreds of protein-protein interactions.

Research highlights

In 2016, the Montoya group published the crystallization of subdomains of two interacting proteins, PICH and BEND3, a complex involved in resolving DNA entanglement between sister chromatids during cell division (Pitchai et al., *Acta Crystallographica. Section F: Struct Biol Commun.*, 2016).

The Program has been involved in a number of collaborative efforts with groups in the Protein Signaling Program where we have provided high quality proteins and in-depth biophysical and structure-function studies. This has resulted in several important papers in the areas of cellular responses to replication stress (Hoffmann et al., *Journal of Cell Biology*, 2016), transcription during class switch recombination (Starnes et al., *Genes & Development*, 2016), and characterizing the binding specificity of a major eukaryotic phosphatase (Hertz et al., *Molecular Cell*, 2016).

International standing and collaborations

The Protein Structure and Function Program collaborates with groups across CPR and CPR researchers extensively use the Platform, which provides proteins for their research and training in protein production. In addition to the groups at CPR, the Program holds international collaborations with groups in UK, Germany, Spain and France. Importantly, we maintain strong connections to the Max IV Synchrotron facility in Lund (Sweden) and the Swiss Light Source (SLS) in Zurich (Switzerland).

In 2016, the Program was one of the founding members of the iSBUC (Integrative Structural Biology at the University of Copenhagen) cluster. iSBUC aims to create optimal conditions for collaboration and sharing of technologies and equipment within the structural biology field. The cluster spans 16 departments at the Faculty of Sciences and the Faculty of Health and Medical Sciences.

"Our objective is to visualize at molecular level the functional details of protein complexes involved in cell cycle proliferation and genome instability."

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
Pablo Alcón Hernández
Diana Kowalik
Danyang Wang

Research Assistant
Saranya Nallapareddy

Technicians
Elisabeth Bragado Nilsson
Kamila Kamuda

Laboratory Assistants
Nika Jachowicz

Also featured in group picture:
Nicholas Egholm Sofos (Postdoc, 2017)

INTERNAL COLLABORATORS

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.

Jiri Lukas (Lukas group, Protein Signaling Program) protein complexes involved in gene duplication.

Jesper V Olsen (Olsen group, Proteomics Program) characterization of kinase substrates.

EXTERNAL COLLABORATORS

Ian Hickson (Center for Chromosome Stability, University of Copenhagen (UCPH), Denmark) chromosome segregation.

Imre Berger (University of Bristol, UK) production of protein complexes.

Christiane Schafitzel (University of Bristol, UK) 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 Maria 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.

2016

Challenges and research aims

The Macromolecular Crystallography group studies the structure of macromolecules involved in 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

A major highlight of 2016 was the ability of program and group leader Guillermo Montoya to win a 60 million DKK (7.8 million EUR) grant from the Novo Nordisk Foundation (NNF) to establish cryo-electron microscopy (cryo-EM) at CPR. The cryo-EM will be placed at the Core Facility for Integrated Microscopy (CFIM) at the Faculty of Health and Medical Sciences and anchored at CPR. It has been a long term goal for CPR to expand its protein technology portfolio with cryo-EM and we are excited to see this goal fulfilled. In 2016, the Macromolecular Crystallography group has established procedures for processing samples for cryo-EM at University of Copenhagen (UCPH) with the help of the CFIM personnel. In addition we have setup the computing protocols to perform cryo-EM processing and the calculation of high resolution structures at CPR.

STRATEGIC GOALS

- To identify how key kinases control mitotic entry and exit by the interplay between kinases and phosphatases
- To establish high resolution cryo-EM techniques for the study of large macromolecular complexes involved in cell proliferation
- To understand the molecular details of genome editing tools to redesign them for biotechnological and therapeutic applications

In 2016, we published the crystallization of subdomains of two interacting proteins, PICH and BEND3 (Pitchai et al., *Acta Crystallographica. Section F: Struct Biol Commun.*, 2016). The complex is involved in resolving DNA entanglement between sister chromatids during cell division. If left unresolved, these entanglements can generate either chromatin bridging or ultrafine DNA bridging in the anaphase of mitosis and the structure of the interacting domains of BEND3 and PICH sheds light on this process. The crystal structure of the BD1 and NTPR domains of PICH and BEND3 provides the first evidence of how a BEND domain interacts with other proteins.

The group published several research articles in 2016, which included collaborations with groups at CPR as well as in Spain. This has resulted in important papers in the areas of cellular responses to replication stress (Hoffmann et al., *Journal of Cell Biology*, 2016), the molecular architecture of human MCM2-7 helicase in complex with nucleotides and DNA (Boskovic et al., *Cell Cycle*, 2016), as well as structural and functional studies of homing endonucleases, which are potential tools for genome modification (Molina et al., *ACS Chemical Biology,* 2016; Prieto et al., *Acta Crystallographica. Section F: Struct Biol Commun.*, 2016).

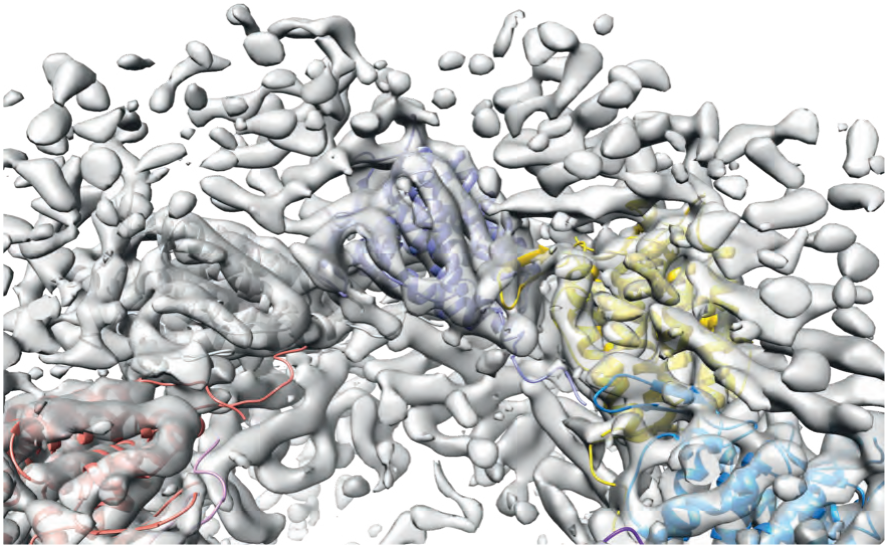


Figure 21 | The figure shows an example of cryo-electron microscopy images generated by the Macromolecular Crystallography group (unpublished data, Pablo Mesa).

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)
Mateo Belluci (Technician)
Motiejus Melynis (Technician)

Eukaryotic Protein Expression
Giuseppe Cazzamali (Team Coordinator)
Alison Lilley (Technician)
Carla Donadoni (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)

The goal of the Protein Expression teams is to provide CPR scientists with state-of-the-art methods within protein expression and protein purification while the Biophysical Protein Characterization team implements activity assays and helps characterize the function of the proteins using biophysical assays. The Platform aims to educate and provide training to CPR scientists in protein expression and characterization while at the same time implement novel methodologies for the use of the CPR scientists.

While the Protein Production and Characterization Platform has previously developed robust protocols to express and purify single domains and small proteins, in recent years these methods were expanded to produce proteins and protein complexes that are involved in key cellular processes for structural and mechanistic studies. In 2016, this challenging enterprise has resulted in the production of protein complexes involved in for example DNA replication (figure 22).

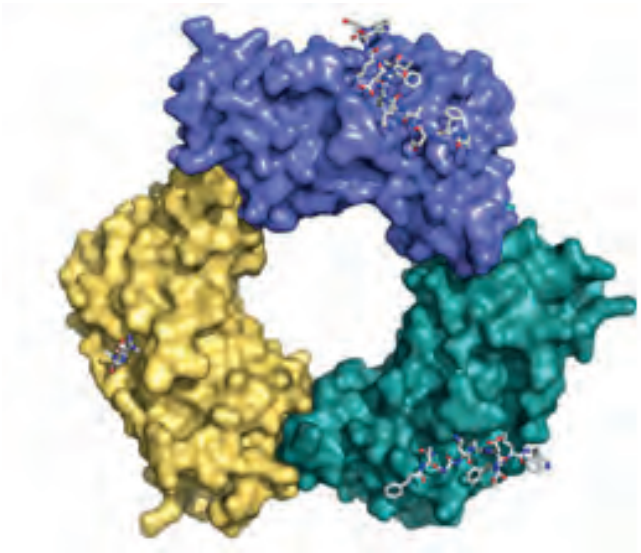


Figure 22 | Crystal structure of a protein complex that protects genome integrity after obstacles to DNA replication as demonstrated in Hoffmann et al., 2016 ('TRAIP is a PCNA-binding ubiquitin ligase that protects genome stability after replication stress', *The Journal of Cell Biology*). The protein complex was produced by the Eukaryotic Expression Team in the Platform and the crystal structure was solved by Guillermo Montoya.

EDUCATION AND CAREER DEVELOPMENT

One of our key missions at CPR is to train, educate, and develop the next generation of protein scientists and in line with this mission we focus strongly on providing excellent opportunities for career development of our junior researchers (postdocs, PhD students, and master/bachelor students) who constitute more than half of our employees. We aim to create an unmatched career development portfolio to attract the most talented young scientists and enable them to reach top international levels early 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 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 to scientific challenges.

"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) to attract exceptional PhD students from around the world to Copenhagen and pioneer a new format for PhD education in Denmark. The program offers talented students holding a university degree from abroad the opportunity do a PhD at one of the NNF research centers. The PhD Program launched in September 2016 with the arrival of fourteen international students, including four at CPR: Lili Niu, Lisa Schubert, Hana Sedlackova and Anamarija Pfeiffer. The students completed three rotation projects in different research groups at CPR before choosing a group to join for their long-term project. At the same time, the students enrolled in a new four-week PhD course, 'Protein Research and Critical Thinking', run by the PhD Program and hosted by CPR. The next round of applications and selection process for the Copenhagen Bioscience PhD program is ongoing at the end of 2016 and four more students are expected to join CPR in September 2017.

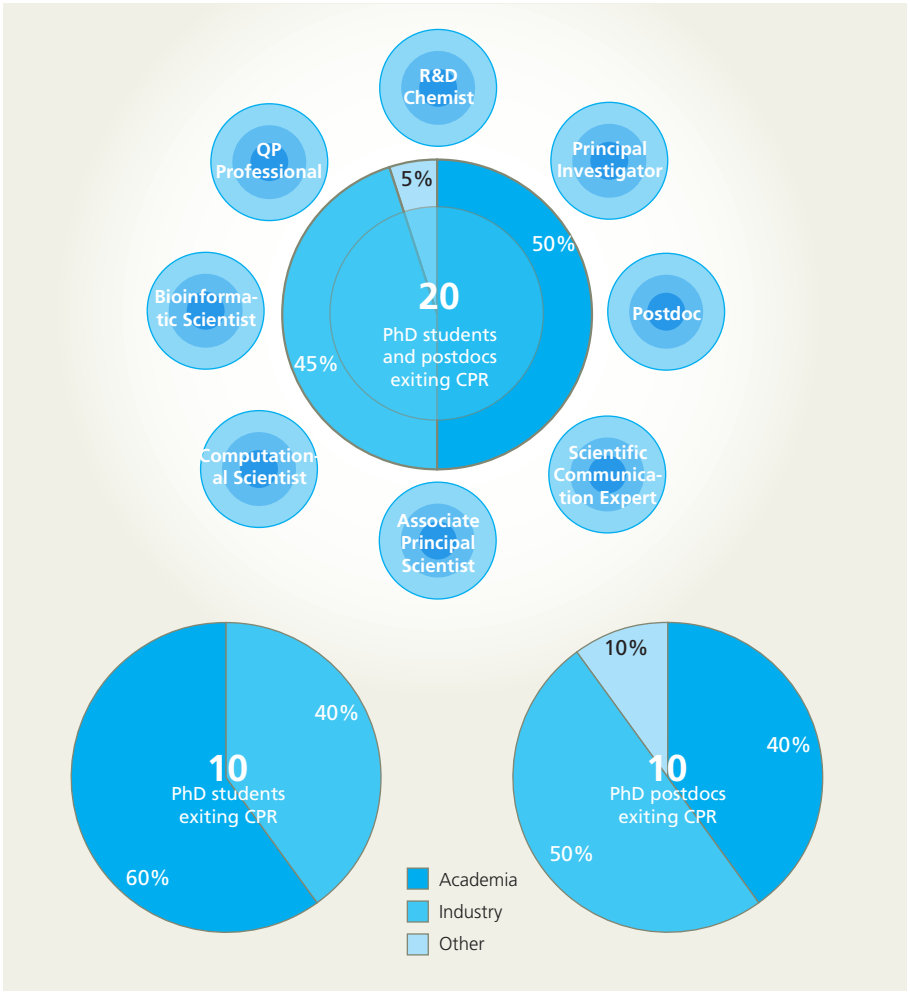


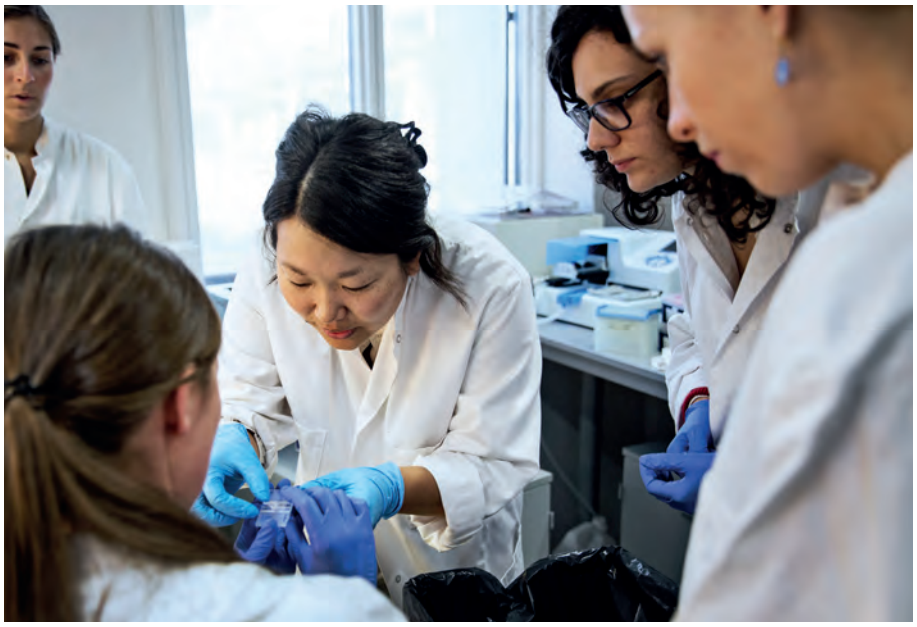
Figure 23 | (top) Positions taken up by PhD students graduating from CPR and postdocs leaving CPR in 2016; (bottom left) Career tracks of PhD students graduating from CPR and (bottom right) postdocs leaving CPR in 2016.

JESPER V OLSEN,
VICE DIRECTOR
DIRECTOR OF EDUCATION

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 move. In 2016, 10 PhD students completed their education here by defending their thesis and moved on to the next stage of their career and in the same period 10 postdocs left CPR. As demonstrated in Figure 23, our young researchers move on to careers both within academia and industry, covering a broad spectrum of scientific positions and 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.

In March 2016, we arranged an 'industry career meeting' for all CPR employees to provide a forum for CPR scientists to build bridges between academic and industrial protein research, identify new areas for collaboration, and identify future job opportunities. The career meeting featured talks by Anders Hinsby, CEO of Orphazyme, and Søren Møller, Managing Investment Director, Novo Seeds. Orphazyme is a start-up biotech company located at COBIS (Copenhagen Bio Science Park) and is a spin-off from the Danish Cancer Society. Anders' talk focused on how to start and run a biotech company. Novo Seeds is one of the most important investors that provide scientific and commercial support to promising early-stage life science projects in Scandinavia and Søren talked about how to get seed money from Novo Seeds for commercialization of biomedical research findings and development of novel technologies within the life sciences.

Another highlight for career development at CPR in 2016 was that CPR together with research institutions in Barcelona, Nijmegen and Milan was awarded a large EU grant of 3.5 million DKK (500.000 EUR) from the H2020 Programme for Science with and for Society, "Celebrating European Science" for the ENABLE project (European Academy for Biomedical Science). As part of ENABLE we have an unprecedented opportunity to support career development of young researchers. The consortium member institutions will each organize one three-day peer-reviewed scientific symposium (CPR is scheduled in 2018) with strong focus on public outreach, scientific activities, public engagement initiatives as well as a career workshop. The ENABLE events are organized by PhD students and postdocs from each member institution and CPR's representatives have a leading role in organizing the career workshops during all symposiums. The main aims of the career workshops are broadening of career horizons, increasing awareness of skill development for young scientists to improve employment chances and creating a platform for vacant (non)-academic positions. A successful kick-off meeting was held in Barcelona in November 2016.



EDUCATIONAL ACTIVITIES

Supervision of students and teaching activities for PhD and master/bachelor students are vital components of CPR's strategy to identify and develop talented students into complete protein scientists. CPR employees at all levels are engaged in teaching activities at the Faculty and elsewhere (Table 1). Taking part in educational activities is an important part of the career development opportunities for CPR 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

Table 1 | Overview of teaching activities 2016

	Type of course	Topic
Proteomics Program	Pre-graduate course, master level	Advanced Methods of the Analysis of Protein Disease Mechanisms (CPR, UCPH)
	Pre-graduate course, master level	Advanced Basic Immunology (UCPH)
	Pre-graduate course, master level	Advanced Protein Science 1 (UCPH)
	PhD course	Mass Spectrometry Coupled to Separation - Techniques in Bioanalytical Chemistry (UCPH)
Disease Systems Biology Program	Pre-graduate course, master level	Introduction to Bioinformatics (UCPH)
	Pre-graduate course, master level	Bioinformatics for Human Biologists (UCPH)
	Pre-graduate course, master level	Obstetric Complications 3rd Trimester (UCPH)
	Pre-graduate course, bachelor level	It og Sundhed - Intro (UCPH)
	PhD course	Bioinformatics Spring Course (Helmholtz Zentrum München, held in Italy)
	PhD course	Integration of Data and Models in Medicine, Germany
	PhD course	Computational Analysis of Protein-Protein Interactions (EMBO, Germany)
	PhD course	Computational Biology (EMBO, Germany)
	Phd course	Computational Microbiology and Microbiome-Based Medicine (Lipari School, Italy)
	Phd course	Network Science (Uppsala University, Sweden)
	Other (pre-graduate level)	Interactive Data Analysis in Python with Pandas using Jupyter Notebook (CBioVikings, Denmark)
	Other (pre-graduate level)	Symposium on Biological Networks (CBioVikings, Denmark)
	Other (pre-graduate level)	Advanced Shell Scripting (CBioVikings, Denmark)
	Other (pre-graduate level)	Galaxy NGS (CBioVikings, Denmark)
	Other (pre-graduate level)	Systems Toxicology (CBioVikings, Denmark)
Protein Signaling Program	Pre-graduate course, master level	Advanced Methods of the Analysis of Protein Disease Mechanisms (CPR, UCPH)
	Pre-graduate course, master level	Cell Cycle Control and Cancer (UCPH)
	Pre-graduate course, master level	Human Genetics (UCPH)
	Pre-graduate course, master level	Principal Subject in Molecular Genetics 2 (UCPH)
	Pre-graduate course, bachelor level	Avanceret Molekylær Biologi (SDU)
	Phd course	Practical Aspects of Protein Purification (CPR, UCPH)
	Phd Course	Protein Research and Critical Thinking (CPR, UCPH)
	Phd course	Statistics for Biologists (UCPH)
	Phd course	Introduction to Molecular Biosciences (UCPH)
	Phd course	EMBO practical course on High-Throughput Microscopy in Systems Biology, European Molecular Biology Laboratory (EMBL, Germany)
	Phd course	High-Throughput Microscopy for Systems Biology (EMBO, Germany)

research activities of the Faculty of Health and Medical Sciences and the wider University of Copenhagen (UCPH).

Educational activities at CPR are coordinated by Vice Director Jesper V Olsen who is responsible for the implementation of CPR-initiated PhD and master level courses at the Faculty, with tasks that range from curricular development to coordination with educational program leaders at UCPH. CPR's engagement in education can be divided into three main themes: student supervision, organization and implementation of master's and PhD courses at UCPH, and teaching and student evaluation at national and international courses.

CPR offers a master course 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. In addition, we offer the course 'Advanced Methods for the Analysis of Protein Disease Mechanisms', organized by Jesper V Olsen with input from most of CPR's Groups and Platforms. In 2016, 'Advanced Methods for the Analysis of Protein Disease Mechanisms' was part of the UCPH summer school program with the aim of recruiting ambitious students both nationally and internationally. Compared to earlier years, the curriculum was modified to include a three-week e-learning module developed in collaboration with UCPH's Centre for Online and Blended Learning. The practical part of the course took place during two weeks in August with 23 participating students. Lectures, e-presentations and laboratory exercises were provided by CPR Faculty, postdocs, PhD students and technicians. The course was rated highly be the participating students who especially valued the hands-on laboratory exercises, journal clubs, and e-lectures, and generally praised the course for providing them with an introduction to, and opportunity to work with, the latest protein technologies.

In addition to CPR-initiated educational activities, CPR researchers contributed to several other educational programs at the Faculty including the pre-graduate courses 'Advanced Basic Immunology' (Jesper V Olsen), 'Advanced Protein Science 1' (Michael L Nielsen) and 'Cell Cycle Control and Cancer' (Jakob Nilsson).

CPR members were actively involved in lecturing in a number of PhD level courses in 2016, in Denmark as well as internationally. For example, Claudia Lukas, Jutta Bulkescher and Fena Ochs were part of the practical courses in High-Throughput Microscopy for Systems Biology at European Molecular Biology Organization (EMBO), Germany, and Lars J Jensen lectured in the EMBO-course on 'Computational analysis of protein-protein interactions: Sequences, networks and diseases' in Hungary.

SEMINARS AND ANNUAL RETREAT

CPR seminar series

The purpose of our seminar series (Table 2) is to attract 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 researchers at CPR and the seminars are always followed by a lunch session between the speaker and a group of interested PhD students and postdocs. This gives our junior staff a unique chance for informal interaction with the speaker where they can ask questions about the science as well as non-scientific aspects such as career-path. After lunch, the speakers are available for sessions with individual researchers.

SPA annual retreat

In September 2016, the second annual retreat of CPR's Student and Postdoc Association (SPA) was held at Hotel Marienlyst in Helsingør and 31 enthusiastic PhD students and postdocs participated in the event. The goal of the retreat is to tighten scientific and social bonds between young CPR scientists, encourage interaction and collaboration across the

center, share knowledge and develop personal skills such as presentation techniques and scientific communication skills. The SPA retreat provides a forum to exchange scientific ideas and discuss innovative solutions to pertinent scientific questions. This was encapsulated in individual short talks and poster presentations on one's research, specialized talks on key scientific techniques at CPR and two overarching interdisciplinary talks that highlighted the close collaborative nature between various research groups at CPR. SPA also had the privilege to host TED speaker Balder Onarheim who challenged participants on notions of scientific creativity. The participants unanimously agreed that the SPA retreat is a great opportunity to meet and informally discuss science with their peers.

Table 2 | Speakers in the CPR Seminar Series 2016

Date	Title	Speaker	Affiliation	Hosted by
Mar 4	The CPEB-family of RNA-binding proteins, mechanisms of action and new functions in cell cycle and cancer	Raúl Méndez de la Iglesia	Institute for Research in Biomedicine (IRB), Barcelona, Spain.	Guillermo Montoya
Mar 4	Modelling malignant growth in Drosophila	Cayetano González	Institute for Research in Biomedicine (IRB), Barcelona, Spain	Guillermo Montoya
Apr 7	Small molecule perturbation of EWS-FLI1 networks: Killing cancer while gaining mechanistic insights	Jeffrey Toretsky	Lombardi Comprehensive Cancer Center (LCCC), Georgetown University, Washington, U.S.A.	Lars Juhl Jensen
Apr 15	From ciliary traffic control to anti-Ras drug candidates	Alfred Wittinghofer	Max Planck Institute of Molecular Physiology, Dortmund, Germany	Guillermo Montoya
May 13	Atomic structure of human y-secretase	Xiaochen Bai	MRC Laboratory of Molecular Biology, Cambridge, UK	Guillermo Montoya
Sep 13	Systems biology analysis of a small organism: what we have learned	Luis Serrano	Centre for Genomic Regulation – Barcelona, Spain	Guillermo Montoya
Sep 20	The Origin Recognition Complex and chromosome inheritance: structure, function and human genetics	Bruce Stillman	Cold Spring Harbor Laboratory, NY, U.S.A	SPA
Sep 22	A patient-centered therapeutic perspective: Sepsis as a clinical model	Pope Moseley	University of New Mexico School of Medicine, US	Søren Brunak
Nov 29	How does a cell decide how to deal with DNA damage?	René H. Medema	Netherlands Cancer Institute, Amsterdam, Netherlands	Jakob Nilsson
Dec 5	High-resolution structure investigation of how proteins recognise chromatin	Fabrizio Martino	The Biological Research Center (CIB) Madrid, Spain	Guillermo Montoya
Dec 14	Structure and mechanism of self-assembling selective autophagy receptors	Arjen Jakobi	EMBL, Heidelberg, Germany	Guillermo Montoya

OUTREACH AND DISSEMINATION

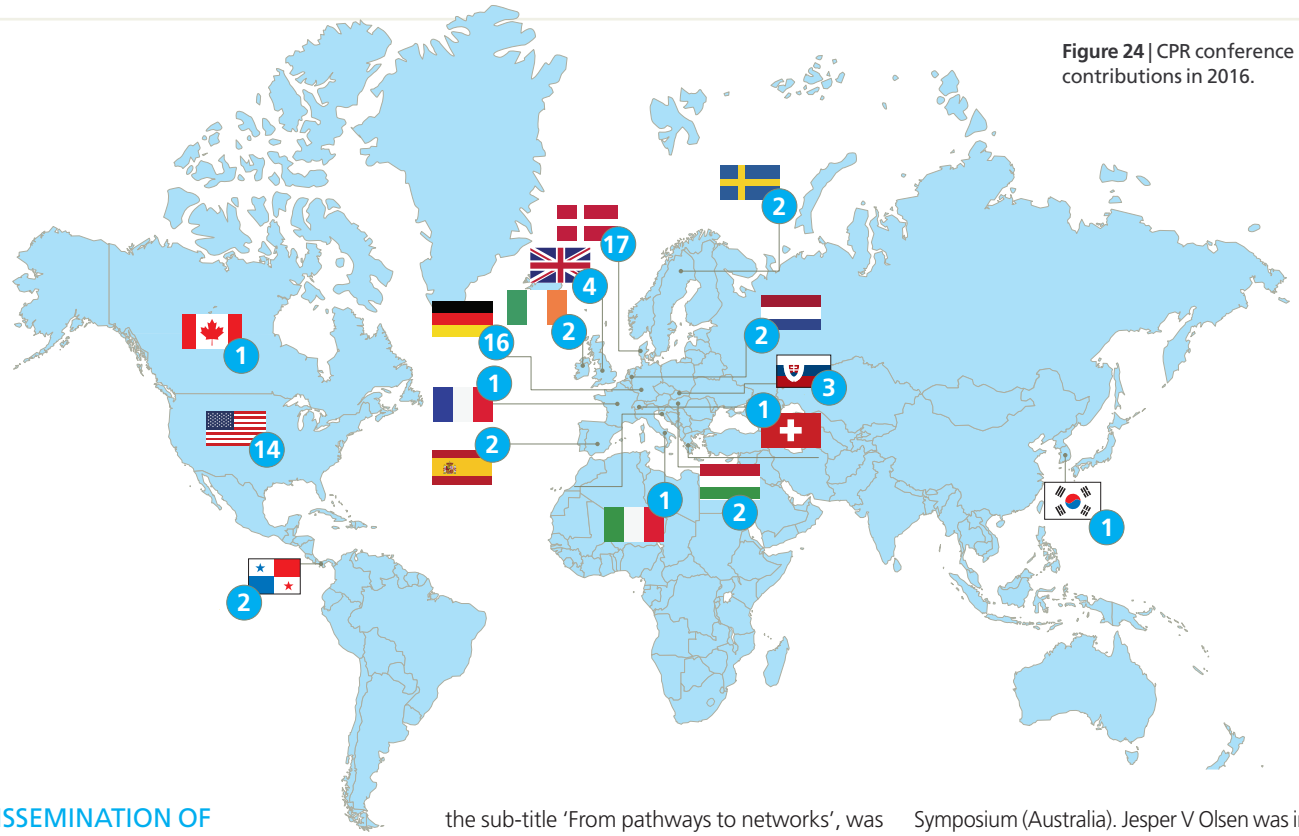


Figure 24 | CPR conference contributions in 2016.

DISSEMINATION OF SCIENTIFIC OUTPUT

In 2016, 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.

A true highlight in 2016 was the third 'Protein Signaling' conference organized by CPR as part of the Copenhagen Bioscience Conference series with generous logistic and financial support from the Novo Nordisk Foundation. Like the conferences in 2012 and 2014, this was an extremely successful and inspiring interdisciplinary meeting that strengthened the position of CPR on the international map of medically-oriented protein science. The conference, with the sub-title 'From pathways to networks', was planned and arranged by Jesper V Olsen, Niels Mailand, Guillermo Montoya, Jakob Nilsson and Lars Juhl Jensen. Also in 2016, Søren Brunak co-organized and hosted the 1st European Conference on Translational Bioinformatics, which was held in UCPH's ceremonial hall and Niels Mailand co-organized the 10th Quinquennial Conference on Responses to DNA Damage: From Molecule to Disease (Egmond aan Zee, The Netherlands).

Scientists from CPR were invited to speak at many high profile conferences (Table 3), which increases the visibility of CPR research and fosters valuable interactions internationally (Figure 24). For instance, Jiri Lukas was invited to talk at diverse conferences in 2016, including the Abcam conference 'Maintenance of Genome Stability' (Panama) and the 'Genomic Instability in Cancer' conference (Berlin, Germany). Among the more than 50 scientific talks that Matthias Mann gave in 2016 were a distinguished lecture at Cancer Research UK (Cambridge University, UK) and the opening talk at the Lorne Proteomics Symposium (Australia). Jesper V Olsen was invited to speak at several conferences in 2016, including the HUPO2016 conference (Taipei, Taiwan) and the 21st International Mass Spectrometry Conference (Toronto, Canada). Søren Brunak was invited speaker at a number of conferences including at the International Society for Computational Biology where he also received the 2016 'Accomplishment by a Senior Scientist Award' for his significant research, education, and service contributions to the field. Finally, from the Protein Structure and Function Program, Guillermo Montoya was key note speaker at the 2016 Current Trends in Biomedicine workshop 'Chaperones in the Maintenance of Cellular Proteostasis' (Baeza, Spain).

Table 3 | Conference contributions

Invited/keynote speaker	117
Poster	47
Organizer	6
Chair	5

MAINTAINING CPR'S IDENTITY

In 2016, we have continued our efforts to establish a strong identity for CPR, both internally and externally, by reaching out to our staff and fellow researchers within our specializations, as well as to our broader academic, industrial, and clinical partners, and the general public. Such outreach is of immense importance for us to maintain our image as an ambitious and internationally renowned research institution.

Collaborations

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

In 2016, we consolidated our status as an unmatched partner in protein science by further strengthening the infrastructure of CPR. The specialized expertise of CPR's well-established technology Platforms, interlinked directly with the research groups, has ensured knowledge and collaboration synergy across CPR as evidenced by our exceptional scientific output. Internally, the Program/Platform structure fosters collaborative research projects 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. In 2016, funding was secured for the addition of a cryo-electron microscopy (cryo-EM) facility in the Protein Structure and Function Program, an expansion that underlines our position as an unmatched partner in protein technological excellence.

CPR fosters collaborative efforts across the Novo Nordisk Foundation (NNF) Center Cluster, with NNF Laureates, and with other institutes and 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, several common projects with Center for Healthy Aging (Ian Hickson), and a collaboration between the Choudhary group and NNF Laureate Stephen Cohen, UCPH, on the function of ubiquitylation in HIPPO signaling. Equally important, we also initiate collaborations with industry – an example of this is the sharing of an Industrial PhD between the Olsen group and Novo Nordisk A/S.

In 2016, CPR continued its clinical interactions, which include Research Director Matthias Mann's collaboration with Bente Klarlund Pedersen at Centre of Inflammation and Metabolism (Rigshospitalet) where Matthias' expertise in plasma proteomics is used for research in exercise and its role in human health. Likewise Søren Brunak and his group members participate in a number of well-established collaborations with researchers from Rigshospitalet, including Anders Perner on intensive care data analysis and Jens Lundgren on big biomedical data analysis on time-ordered comorbidities.

CPR is part of the national PRO-MS consortium, which is supported by 40 million DKK (5.3 million EUR) from the Danish Ministry of Higher Education and Science. The aim of the project is to establish a national research infrastructure on mass spectrometry for functional protein research and proteomics and is a collaboration between Danish proteomics and mass spectrometry laboratories located at University of Southern Denmark, University of Copenhagen, Aarhus University, Aalborg University and Technical University of Denmark. Jesper V Olsen is part of the steering committee that coordinates all PRO-MS activities at the partner universities.

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

LOTTE SKIPPER,
COMMUNICATION CONSULTANT

Internal communication

CPR's identity 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. We use CPR-wide seminars and meetings as a means to enforce knowledge-sharing and internal communication, building and supporting a feeling of identity among our staff. Every year we host four CPR Center meetings where the management conveys important information to CPR staff, we celebrate successes and people, and knowledge-share via conceptual talks given by CPR's program-, group- and platform leaders. Equally, our bi-weekly internal research seminars, CPR Research in Progress, where junior researchers present their research and are challenged by their peers and senior researchers, play an important role in nurturing relations between CPR researchers to maintain a high level of integration across CPR's research environment further fostering cross-disciplinary projects and collaborations. We believe that these internal meeting series help create an improved understanding of the concepts of 'who we are' (CPR as organization), 'why we are here' (CPR's vision and mission) and 'what we do'.

In 2016, we have taken steps to improve 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 all our 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.

MEDIA COVERAGE AND PUBLIC ENGAGEMENT

We continuously strive to raise the profile of the CPR brand by generating high-quality scientific results and disseminating these through CPR's website, the Faculty's 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 developing CPR as a hub not only for protein science but also for communication about protein science. In 2016, we made great progress in this area, and published 25 press releases and other news stories on our website, covering breakthrough research, grants, and awards won by CPR staff and other major developments, such as the establishment of the cryo-EM facility under Guillermo Montoya's research program. In addition, we have continued our efforts to profile our research leaders towards the general public by producing interesting portraits that convey in laymen terms the topic of their research field and the reasons that they first became fascinated with it. These portraits are produced in collaboration with the Communication Department at the Faculty of Health and Medical Sciences (UCPH).

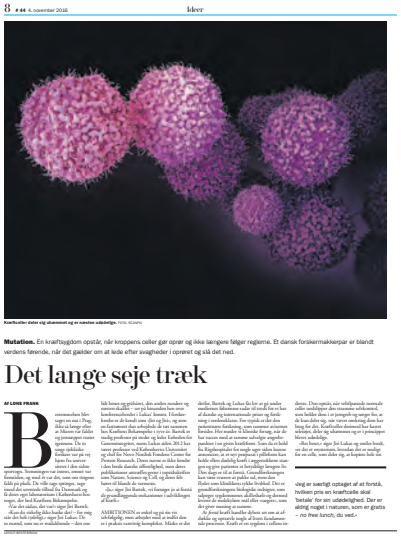
Our most exciting news stories and our research leader portraits are distributed via the Faculty communication department to influential scientific journalists, and signposted using CPR's social media platforms and as news content on our website. Our news stories are also disseminated via twitter and CPR's Facebook and/or LinkedIn pages.

Via the Faculty's network of science journalists we disseminated some of our news stories to specific news media both in Denmark and internationally. An example of a story that received excellent coverage was when CPR Executive Director Jiri Lukas was awarded the prestigious Grand Nordic Prize from the Fernström Foundation for his groundbreaking research on cancer. He shared the prize with his long-term colleague Professor Jiri Bartek from the Danish Cancer Society. The story about the two researchers' research and extraordinary collaboration spread from the Fernström Foundation and the Faculty of Health and Medical Sciences to a number of Danish and Swedish newspapers topped with a profile article presenting Jiri Lukas and his research in the Danish weekly newspaper Weekendavisen (Figure 25).

Also in 2016, Research Director Søren Brunak featured in a Danish Broadcasting (DR) series 'Sygdom Søges' that offered a critical review of genetic profiling tests offered by private companies to the general public. In the program, Søren Brunak did not recommend the use of genetic profiling for healthy individuals in the foreseeable future. Instead he predicted that 'in 10 years from now, these types of tests will be useful in a clinical setting, to decide on treatment regimens for diseased individuals'.

Our researchers play an important role in representing CPR on the local and international science scene presenting their research and spotlighting CPR's unique organization with highly specialized research programs and technological platforms. However, public engagement and outreach to the general public are also important priorities for us. In this role CPR participates in the ENABLE project where members of CPR's Student and Postdoc Association have succeeded to bring substantial funds (800.000 DKK, 0.1 million EUR) to CPR by responding to the Horizon2020 call 'Science with and for Society' together with three other renowned European research institutes to establish the European Academy for Biomedical Science (ENABLE) designed to promote international networking and career development of young scientists. ENABLE will organize five annual three-day scientific symposia and actively seek public engagement via outreach activities to the European public, as well as to primary- and secondary-school children. Another strong element of the symposia will be specific career workshops covering essential skills for widening the career paths for young scientists. The ENABLE kick-off event took place in Barcelona in 2016 and CPR will host the 2018 symposium.

In 2016, CPR reached out to talented Danish secondary school students in the Science Talent program to create awareness about CPR's research for the wider public. CPR collaborates with the 'Science Talent Academy', a learning program for scientifically talented upper secondary school students from Denmark. A team of junior researchers organized a Protein Camp day full of research activities for 62 highly talented young Danish students. During the day, the students were introduced to research activities at the center and tested a series of methods in areas covering all CPR research programs.



Mutation. En kraftsygdom opstår, når kroppens celler gør oprør og ikke længere følger reglerne. Et dansk forskermakkerpar er blandt verdens førende, når det gælder om at lede efter svagheder i coprået og slå det ned.

Det lange seje træk

AF LONE FRANK

Figure 25 | Profile article presenting Jiri Lukas and his research in the Danish weekly newspaper Weekendavisen.

RESEARCH GRANTS AND AWARDS

FINANCIAL STRATEGY

Since its founding in 2007, CPR has been awarded a total of 780 million DKK (104 million EUR) from the Novo Nordisk Foundation (NNF). 600 million DKK was awarded for the first funding period, 2008-2017. In 2014, CPR was awarded additional 180 million DKK (24.2 million EUR) and the grant period was extended until the end of 2019. CPR aims to maintain an annual turnover in the 2015-2019 NNF funding period of at least 130 million DKK (17.3 million EUR), with the NNF contribution making up 40% or more of the total funding for CPR.

OVERVIEW OF FUNDING SECURED

In 2016, CPR's turnover amounted to 154.1 million DKK (20.6 million EUR). Of the turnover in 2016, NNF's contribution was 67 million DKK (8.9 million EUR), 83 million DKK (11 million EUR) was provided as other external funding, and the residual turnover came from the University of Copenhagen (UCPH). The 2016 turnover was at an expected higher level than previous years, since 42.9 DKK million (5.7 million EUR) was spent on equipment.

CPR's strategies for attracting funding were highly successful in 2016 (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 (EUR 9.3 million), in 2015 the total amount of external funding had risen to 180 million DKK (24 million EUR), and in 2016 the value was 120 million DKK (16 million EUR). Of particular note, 60 million DKK (8 million EUR) was granted by NNF to Research Director Guillermo Montoya to establish cryo-electron microscopy at CPR.

Our scientists have succeeded in securing funding from many different sources in 2016 (Table 4). CPR's new group, the Duxin group

Table 4 | Funding secured in 2016

Funder	Project	Recipient	Type	million DKK	million EUR
Danish Cancer Society	New factors in DNA double-strand break repair and genome stability maintenance	Claire Aline Guerillon	Danish private grant	2.1	0.3
Danish Cancer Society	Deciphering a novel class of cancer mutations in BRCA2	Jakob Nilsson	Danish private grant	0.2	0.03
Danish Cancer Society	Unraveling the functional role of protein arginine methyltransferases PRMT1 and CARM1 on growth factor-independent ERK activation in cancer cells	Michael Lund Nielsen	Danish private grant	2.1	0.3
Lundbeck Foundation	Regulation of DNA-protein crosslink repair in human cells	Niels Mailand	Danish private grant	1.6	0.2
Lundbeck Foundation	Expanding the histone code for DNA repair and beyond	Niels Mailand	Danish private grant	9.2	1.2
Novo Nordisk Foundation	MicrobLiver	Matthias Mann	Danish private grant	8.2	1.1
Novo Nordisk Foundation	Copenhagen Bioscience PhD Programme 2016	Jiri Lukas	Danish private grant	1.8	0.2
Novo Nordisk Foundation	Cryo Electron Microscopy Programme	Guillermo Montoya	Danish private grant	60.0	8.0
Novo Nordisk Foundation	In-depth analysis and characterization of the mammalian lysine methylome	Jesper Velgaard Olsen	Danish private grant	1.4	0.2
Novo Nordisk Foundation	Defining PP2A-B55 substrate specificity	Jakob Nilsson	Danish private grant	1.4	0.2
Novo Nordisk Foundation	Molecular phenotypic profiling of protein arginine methyltransferase 5 (PRMT5) in cancer cells by high-throughput quantitative mass spectrometry	Michael Lund Nielsen	Danish private grant	1.4	0.2
Stadslæge Svend Ahrend Larsen og Grosserer Jon Johannessons Fond	Nedregulering af Huntington ved RNA-interfrekvens. en strategi for behandling af Huntingtons Chorea	Niels H Skotte	Danish private grant	3.1	0.4
Danish e-Infrastructure Cooperation (DeIC)	ActionableBiomarkersDK	Søren Brunak	Danish public grant	0.4	0.1

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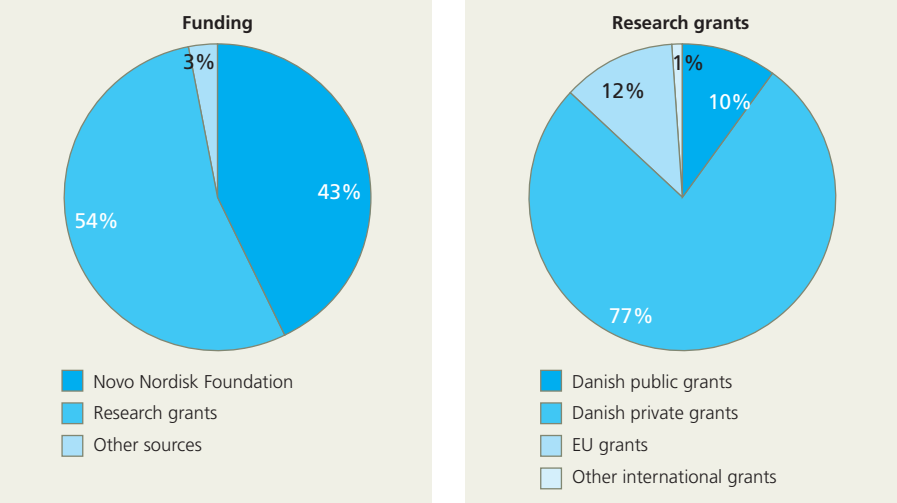

Figure 26 | (left) Breakdown of Novo Nordisk Foundation vs. other funding; (right) breakdown of the type of research grants awarded.

Table 4 (continued)

Funder	Project	Recipient	Type	million DKK	million EUR
Danish Council for Independent Research, Medical Sciences	Identification and functional characterization of factors and mechanisms that coordinate helicase and polymerase activities under mild DNA replication stress	Kumar Somyajit	Danish public grant	2.2	0.3
Innovation Fund Denmark	Regulating protein ubiquitylation as a new treatment principle for Neurodegenerative diseases - Exploring a new enzyme class as drug target	Niels Mailand	Danish public grant	0.4	0.1
Danish Agency for Institutions and Educational Grants	Danish National Mass Spectrometry Platform for Functional Proteomics (PRO-MS)	Jesper Velgaard Olsen	Danish public grant	8.5	1.1
EU - Coordination & Support Action (CSA)	European Academy for Biomedical Science (ENABLE)	Jiri Lukas	EU grant	0.8	0.1
EU - European Research Council (ERC)	Mechanism of DNA-protein cross-link repair in S phase (DPC REPAIR)	Julien Duxin	EU grant	11.2	1.5
EU - Innovative Medicines Initiative (IMI)	Real World Outcomes across the AD spectrum for better care: Multi-modal data Access Platform (ROADMAP)	Søren Brunak	EU grant	1.9	0.3
EU - Personalising Health and Care (PHC)	Gut-and-liver axis in alcoholic liver fibrosis (GALAXY)	Lars Juhl Jensen	EU grant	1.0	0.1
European Molecular Biology Organazation (EMBO)	EMBO Long-Term Fellowship	Ivo Alexander Hendriks	International grant	0.3	0.0
The Regents of the University of New Mexico (NIH Subaward)	Illuminating the Druggable Genome	Søren Brunak	International grant	1.0	0.1
Eric K. Fernströms Stiftelse	Fernström Grand Nordic Prize	Jiri Lukas	Prestigious award		

(Protein Signaling Program) secured 11.2 million DKK (1.5 million EUR) for the European Research Council (ERC) Starting Grant project 'Mechanisms of DNA-protein cross-link repair in S phase (DPC REPAIR)'. Søren Brunak's group (Disease Systems Biology Program) secured 1.9 million DKK (0.3 million EUR) in funding for the EU project 'Real World Outcomes across the AD spectrum for better care: Multi-modal data Access Platform (ROADMAP)'. This project has 23 partners from both Europe and the US. In addition, Jesper V Olsen (Proteomics Program) received 8.5 million DKK (1.1 million EUR) for the grant 'Danish National Mass Spec trometry Platform for Functional Proteomics (PRO-MS)' from The Danish Agency for Institutions and Educational Grants (Ministry of Higher Education and Science). The grant is part of the national PRO-MS consortium that is supported by 40 million DKK (5.3 million EUR) to establish a national research infrastructure on mass spectrometry for functional protein research and proteomics.

SCIENTIFIC OUTPUT: BIBLIOMETRICS

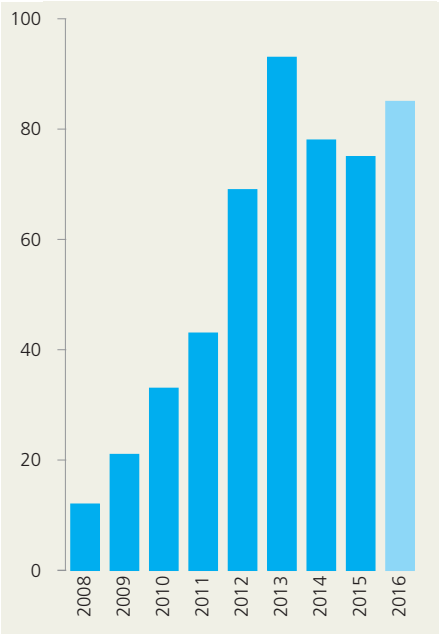

Figure 27 | In 2016, 87 papers were published by CPR scientists.

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

Position	Name	Cited publications	Citations	h-index
ED, RD, GL	Jiri Lukas	197	26,703	90
VD, GL	Jesper V Olsen	126	15,954	60
RD, GL	Søren Brunak	243	38,249	81
GL	Chunaram Choudhary	52	4,433	34
GL	Jeremy Daniel	24	1,844	20
GL, PL	Lars J Jensen	138	14,554	57
PL	Claudia Lukas	47	8,316	36
GL, PL	Niels Mailand	62	4,968	37
RD, GL	Matthias Mann	564	113,079	172
RD, GL, PL	Guillermo Montoya	91	2,368	28
GL, PL	Michael L Nielsen	68	4,783	34
GL	Jakob Nilsson	45	1,371	18
GL	Julien Duxin	7	277	7

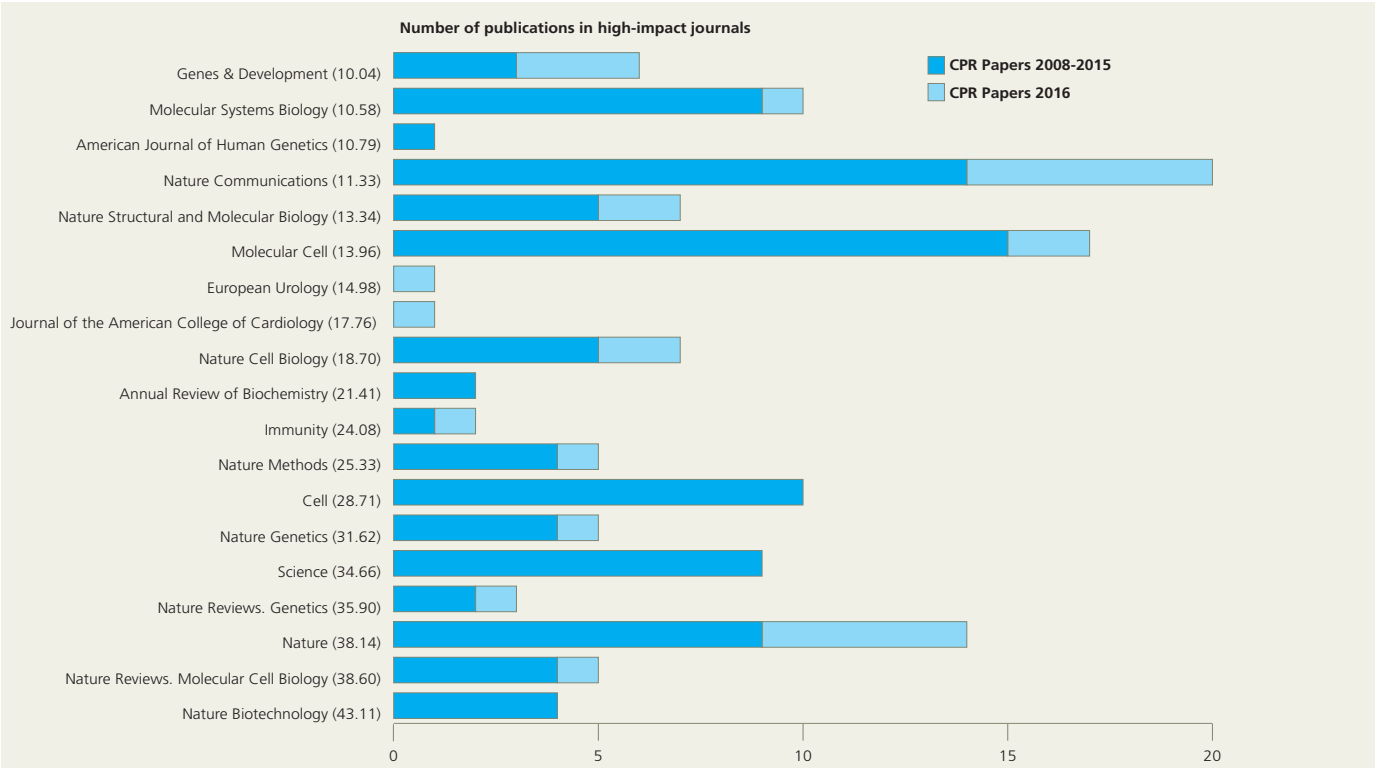

Figure 28 | CPR Papers published in journals with impact factor ≥10 (2-year impact factors from 2015 given in brackets. Source: Web of Science). In 2016, 26 of CPR's papers were published in journals from prestigious publishing houses such as *Nature*, *Science* and *Cell*.

SCIENTIFIC OUTPUT: 2016 PUBLICATIONS

CPR authors are highlighted in bold. Ordered alphabetically by first author surname. Includes papers published online ahead of print in 2016.

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

Mass-spectrometric exploration of proteome structure and function
Aebersold, R & **Mann, M**. Nature, vol 537, no. 7620, pp. 347-55.)

SDCCAG8 Interacts with RAB Effector Proteins RABEP2 and ERC1 and Is Required for Hedgehog Signaling
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Standardized benchmarking in the quest for orthologs
Altenhoff, AM, Boeckmann, B, Capella-Gutierrez, S, Dalquen, DA, DeLuca, T, Forslund, K, Huerta-Cepas, J, Linard, B, Pereira, C, Pryszcz, LP, Schreiber, F, da Silva, AS, Szklarczyk, D, Train, C-M, Bork, P, Lecompte, O, von Mering, C, Xenarios, I, Sjölander, K, **Jensen, LJ**, Martin, MJ, Muffato, M, Gabaldón, T, Lewis, SE, Thomas, PD, Sonnhammer, E, Dessimoz, C & Quest for Orthologs consortium. *Nature Methods*, vol 13, pp. 425-30.

Offline High pH Reversed-Phase Peptide Fractionation for Deep Phosphoproteome Coverage
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Diagnosis trajectories of prior multi-morbidity predict sepsis mortality
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Temporal order of disease pairs affects subsequent disease trajectories: the case of diabetes and sleep apnea
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Metaproteomics of saliva identifies human protein markers specific for individuals with periodontitis and dental caries compared to orally healthy controls
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