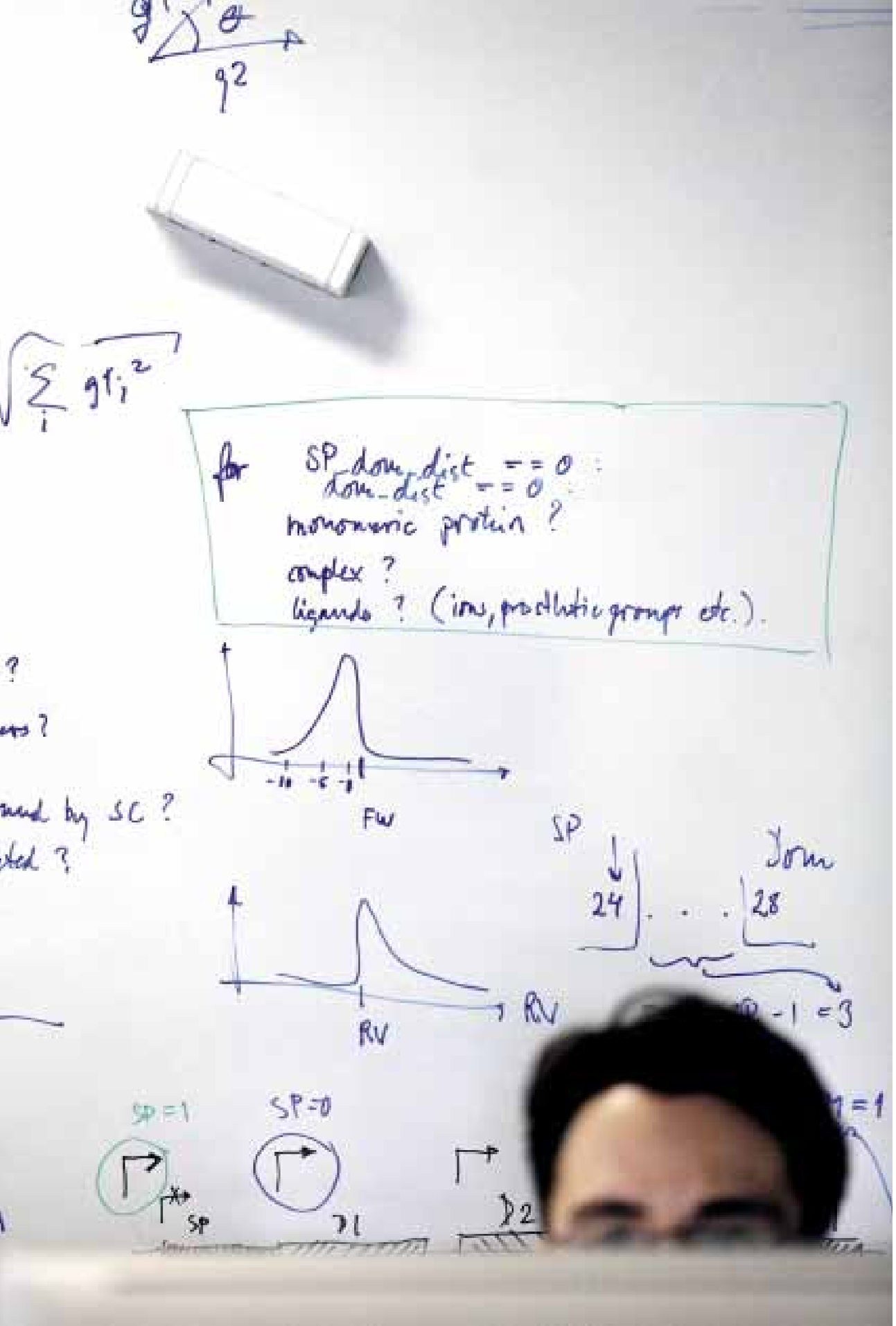
The Novo Nordisk Foundation
Center for Protein Research

Annual Report 2011



Contents

Progress and Performance in 2011

Another successful year for the Novo Nordisk Foundation Center for Protein Research has now passed. During the year, we further consolidated our operations, and added many excellent staff members to our research departments and to the Facility for Protein Science and Technology. Since CPR continuously strives to be at the leading edge within our focus areas, we are constantly looking for the best talent to join our team.

In order to be a world-leading research organization, it is extremely important that we create a flexible, dynamic and yet challenging work place. One of the important parameters in this respect is to have a highly international work environment. We are proud that the Center now houses scientists and support staff representing 25 different nationalities, joining us from countries not only in northern Europe but all around the globe. Among the scientific staff, nearly half have an international background. These CPR members come to us with their diverse backgrounds and with training from top laboratories, making them ready to 'hit the floor running'. This internationalization has immensely increased our capacity at this more mature operational stage, even as compared to 2010. Importantly, this scientific talent base adds strength to the general research community in that CPR was the first center in Denmark supported by the Novo Nordisk Foundation and it has now been joined by several other centers focusing on metabolic, biosustainability and stem cell research.

To further strengthen CPR, in 2011 we recruited two new group leaders in our Disease Biology Department, Assoc. Prof. Jakob Nilsson (from the Biotech Research and Innovation Centre, BRIC, Copenhagen) and Assoc. Prof. Jeremy Daniel (from the NIH, Bethesda, US). Jakob started at CPR during autumn, and Jeremy will join our Center in early 2012. We wish them both the best of luck with their new positions here.

The Center has further upgraded the research infrastructure, for instance with a highly improved mass spectrometry machine park, probably one of the best set-ups world-wide. We have also successfully implemented and improved our protein production platform so that we now have the capacity to express native and full-length proteins in human cells. These are just two examples amongst many significant infrastructure upgrades that the Center has made during 2011.

We continue to work with the best laboratories worldwide, in the areas of Disease Biology, Proteomics, Computational Biology, Chemical Biology and Protein Biotechnology. New, important collaborations started during the year include the Center for Healthy Ageing (University of Copenhagen, Denmark), Lund University (Sweden), the Broad Institute of MIT and Harvard (Boston, US) and the University of Cambridge (UK).

Frequent staff exchanges are also becoming increasingly routine, further promoting cross-fertilization of ideas, experience and creativity.

2011 was the most productive year so far with more than 50 published or accepted peer-reviewed scientific papers appearing in a number of the highest impact journals. We had several papers in Science, Nature Cell Biology and Science Signaling.

Finally, as the Center now approaches its third (truly) operational year since the official inauguration of the laboratories in 2009, we are starting to see staff members moving on to new challenges, after having significantly contributed to the Center's activities as part of their training. We are very happy to see some of our alumni moving to new academic and industrial careers, e.g. our postdoctoral fellow Erik Vernet who joined Novo Nordisk A/S as a research scientist in 2011.

After the establishment phase of the Center, I too have decided to move on to new challenges, and after almost ten years abroad, I have left the Center to take up a new role back home in Stockholm. However, I leave the Center in very capable hands, as Prof. Jesper Velgaard Olsen has accepted to take on the role as interim Managing Director, while the recruitment for my replacement is ongoing.

CPR is now excellently positioned with highly skilled staff and research resources, and will for sure continue to develop to become an even more exciting work environment. I am very grateful for the time I spent with the Center and its staff members, and will continue to follow its development in the years to come, although now from a more remote location.

Michael Sundström
Managing Director
Until September 30, 2011

Jesper Velgaard Olsen
Interim Managing Director and Group Leader
From October 1, 2011

Christopher Austin, Chairman of the SAB - on the Rapid Establishment of CPR

The Scientific Advisory Board is impressed with the outstanding scientific achievements of CPR over the last year, the substantial progress in securing competitive grants to complement the funding from the Novo Nordisk Foundation and the outreach to the local and international scientific community.

CPR is unique in its infrastructure and its combination of world-class protein production, proteomics, and disease biology to apply these technologies to important problems in biology and medicine. Having completed its start-up phase, CPR is now poised to reach its potential as an international leader in protein science. Very strong foundations of staffing, infrastructure, and basic science productivity established by CPR in the last four years, will move the Center to the next level of productivity and impact, emphasizing cross-center collaboration and translational aspects of protein science.

The management of CPR and Ulla Wewer, Dean of the Faculty of Health Sciences, are to be commended for their vision in conceptualizing, and effectiveness in implementing, this unique organization.

Christopher Austin, Chairman of the SAB
Director of the NIH Chemical Genomics Center
Bethesda, Maryland, US

Boards

The Center greatly appreciates the advice on general and strategic matters provided by our Scientific Advisory Board (SAB) and Steering Committee. The members of our SAB met during a two-day site visit to CPR in May 2011, with presentations and face-to-face discussions with group leaders, PhD students and postdoctoral fellows as part of the program. We are fortunate to receive guidance and feedback on our scientific progress and set-up from such experienced, expert scientists. The SAB is designed to rotate frequently and Dr. Peer Bork and Prof. Ray Stevens stepped down in May 2011. We would like to extend our gratitude to Peer and Ray for their time and commitment. The Community Target Committee (CTC) continues to assist the Facility for Protein Science and Technology with evaluating external collaborative project proposals. Anders Lund stepped down from the CTC during the year; however, we are happy to welcome Assoc. Prof. Claus Storgaard as a new member.

Scientific Advisory Board

- **Dr. Christopher Austin,** Director of the NIH Chemical Genomics Center (NCGC), Bethesda, Maryland (US) (Chair)
- **Dr. Peer Bork,** Joint Head of Unit, Senior Scientist and Strategic Head of Bioinformatics, EMBL Heidelberg (Germany) (reaching end of term during 2011)
- **Prof. Ivan Dikic,** Director of the Institute of Biochemistry, Goethe University, Frankfurt and Scientific Director of the FMLS/Frankfurt Institute for Molecular Life Sciences (Germany)
- **Dr. Anthony A. Hyman,** Group Leader and Director at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden (Germany)
- **Prof. Poul Nissen,** Principal Investigator at the Department of Molecular Biology, Centre for Structural Biology, Aarhus University (Denmark)
- **Prof. Tony Pawson,** Senior Investigator at the Samuel Lunenfeld Research Institute, Toronto (Canada)
- **Dr. Pernille Rørth,** Research Director, Institute of Molecular and Cell Biology, A-STAR (Singapore)
- **Prof. Ray Stevens,** Professor and Group Leader at the Departments of Molecular Biology and Chemistry, The Scripps Research Institute, San Diego (US) (reaching end of term during 2011)
- **Prof. Torben Falck Ørntoft,** Head of Department of Molecular Medicine, Aarhus University Hospital at Skejby (Denmark)

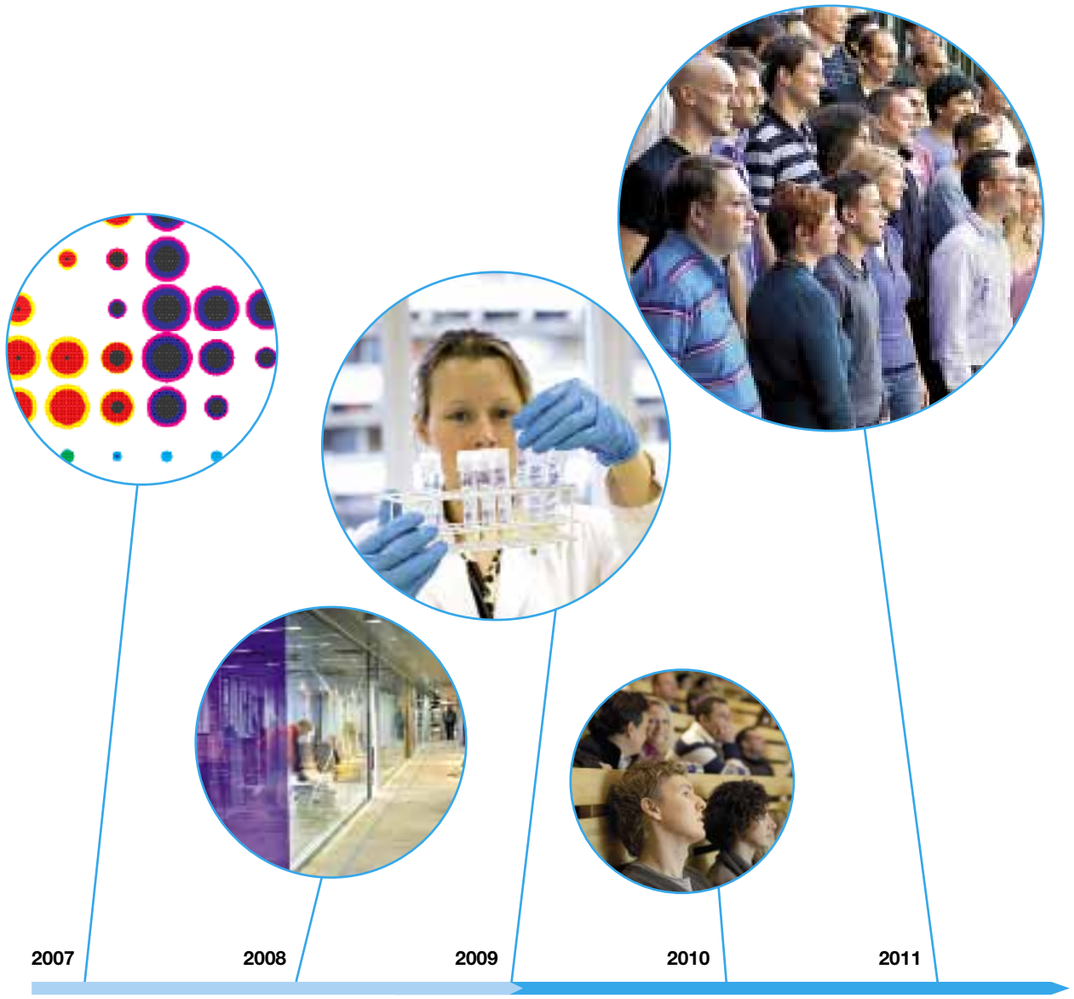
Steering Committee

- **Nils O. Andersen,** Dean of the Faculty of Science, University of Copenhagen
- **Per Holten Andersen,** Dean of the Faculty of Life Sciences, University of Copenhagen
- **Anders Bjarklev,** Prorector of the Technical University of Denmark
- **Ulla Brockenhuus-Schack,** Managing Director, Seed Capital
- **Ingemar Carlstedt,** Assistant Dean of the Faculty of Medicine, Lund University
- **Leif Beck Fallesen,** Chairman of the Copenhagen Business Task Force
- **Sven Frøkjær,** Dean of the Faculty of Pharmaceutical Sciences, University of Copenhagen
- **Lars Goldschmidt,** Managing Director, Danish Association of Consulting Engineers
- **Jannik Hilsted,** Medical Director, Copenhagen University Hospital
- **Kim Høgh,** Group Managing Director, Capital Region of Denmark
- **Ida Sofie Jensen,** Managing Director, Danish Association of the Pharmaceutical Industry (LIF)
- **Mathias Uhlén,** Professor, Royal Institute of Technology (KTH), Stockholm
- **Ulla Wewer,** Dean of the Faculty of Health Sciences, University of Copenhagen (Chair)

Community Target Committee

- **Dr. Peter Andreasen,** Department of Molecular Biology, Aarhus University
- **Prof. Lars Björck,** Department of Clinical Sciences, Division of Infection Medicine, Lund University
- **Prof. Henrik Ditzel,** Center for Medical Biotechnology, University of Southern Denmark, Odense
- **Dr. Michael Toft Overgaard,** Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University
- **Prof. Ole William Petersen,** Department of Medical Anatomy, University of Copenhagen
- **Prof. Thue Schwartz,** Institute for Neuroscience and Pharmacology, University of Copenhagen
- **Assoc. Prof. Claus Storgaard,** Copenhagen Biocenter, Biotech Research and Innovation Centre, University of Copenhagen
- **Prof. Kristian Strømgaard,** Department of Medicinal Chemistry, University of Copenhagen
- **Prof. Birte Svensson,** Institute for Systems Biology, Enzyme and Protein Chemistry, Technical University of Denmark
- **Assoc. Prof. Mats Wikström,** The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen (Chair)

From Start-up to Full-scale Operations



2007

staff 4

- Announced in April
- Recruitment of senior staff
- Design and planning of laboratories

2008

staff 9

- Re-building of laboratories (Jan)
- Projects initiated at remote laboratories (peaked at 13 locations)
- Additional Group Leaders and core of Admin & Support staff recruited

2009

staff 55

- Full-scale recruitment campaign initiated
- Lab inauguration (June)
- Re-location of remote CPR staff to labs (June-Sept)
- Core of infrastructure established

2010

staff 107

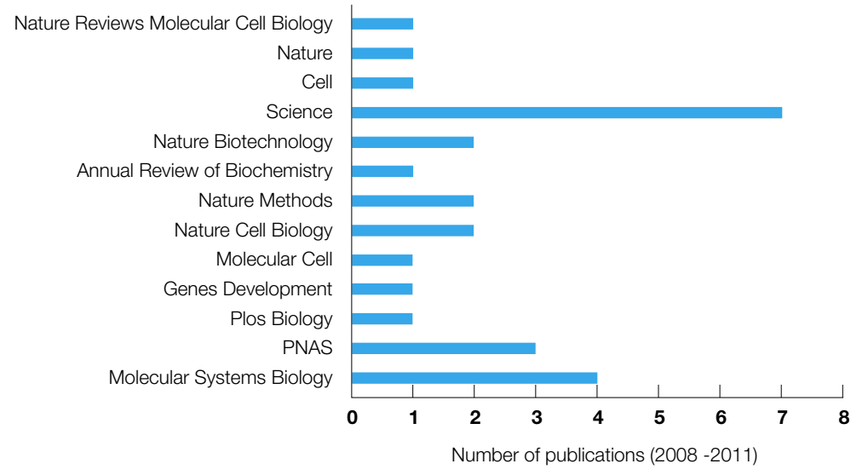
- Rapid expansion (staff, research infrastructure)
- Focus on research projects, publications, dissemination of data
- Collaborative network & projects
- External grants to supplement core funding

2011

staff currently >130

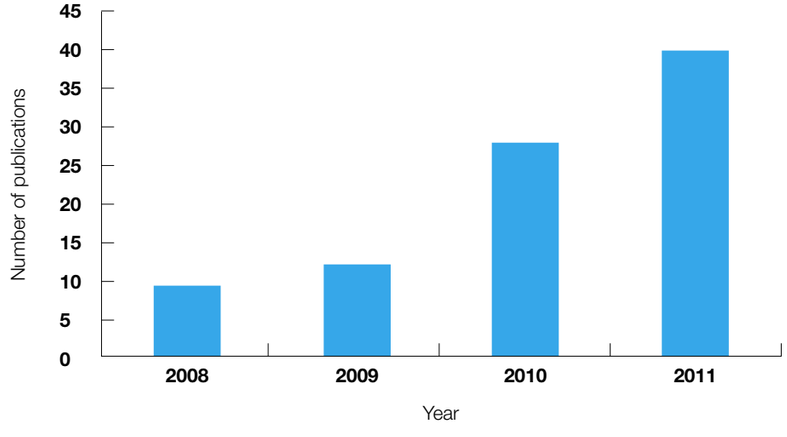
- Full-scale operations
- Recruitment of Group Leaders completed
- Latest generation high-end mass spectrometry and microscopy instrumentation acquired
- More than 50 scientific publications

Number of Publications in High Profile Journals



Based on all CPR publications in Web of Science from 2008-2011; average impact factor ten or more.

Number of Publications per Year



Based on all CPR publications in Web of Science.

Department of Disease Biology

A general infrastructure upgrade (amounting to DKK 10 million) was planned and approved during the year. In particular, this included the acquisition of a suite of state-of-the-art microscopes, allowing researchers at CPR to follow medium- to high-throughput siRNA-based screens, as well as cutting-edge time-lapse and confocal microscopy. In addition to the acquisition of capital equipment, our cell culture facilities were expanded substantially, enabling us to accommodate the growing needs for tissue culture work resulting from the expansion of the department.

Honorary Professor at CPR

We are very pleased that Prof. Jesper Svejstrup has been associated with the Faculty of Health Sciences as Honorary Professor since February 1, 2011. In this role he will provide advice for research efforts at CPR and at the Department of Cellular and Molecular Medicine (ICMM). Prof. Svejstrup heads the Mechanisms of Gene Transcription Laboratory at the Cancer Research UK London Research Institute, and is a leader in research areas of profound importance for both CPR and ICMM. At CPR, Prof. Svejstrup will mentor the young group leaders in the Department of Disease Biology, providing guidance on matters such as research strategies, leadership and career development.

Chromatin Structure and Function

Group Leader, Assoc. Prof. Jeremy Daniel

The Chromatin Structure and Function research group will start activities in the Center on January 1, 2012. Planning has taken place on site and the group has already initiated in-house collaboration with Jesper Velgaard Olsen's group in the Department of Proteomics. In addition, the group has begun recruitment of personnel.

The mission of our laboratory is to identify therapeutic targets for cancer by investigating basic mechanisms for how dynamic chromatin environments impact the stability of our genomes. DNA double-strand breaks (DSBs) can be caused by exogenous damage or collapsed replication forks. They also transiently occur during normal physiology as part of e.g. programmed DNA rearrangements in lymphocytes. Once damaged, a DNA lesion such as a DSB must first be made accessible by changes in chromatin structure to allow for subsequent DNA repair. Recently, Jeremy identified the first histone-modifying complex that directly controls chromatin accessibility critical for lymphocyte rearrangements, findings that were published and highlighted in Science Magazine.

Despite our understanding of the role of chromatin in transcriptional regulation, the consequences of changes in chromatin structure and post-translational modifications that directly affect the stability of our genomes to suppress cancer remain surprisingly unclear. However, recent genome, exome, and RNA sequencing efforts have established that a number of post-translational histone modifiers are significantly mutated in cancer. Our approach is to utilize mice as a physiological model, together with a combination of biochemistry, flow cytometry, genomics, and proteomics, to investigate how protein complexes, that modify or associate with chromatin, regulate genomic stability and prevent lymphoid malignancy. We are also interested in understanding how transcription and DSB repair can influence each other and how this coordination occurs within the context of chromatin.

Mitotic Mechanisms and Regulation

Group Leader, Assoc. Prof. Jakob Nilsson

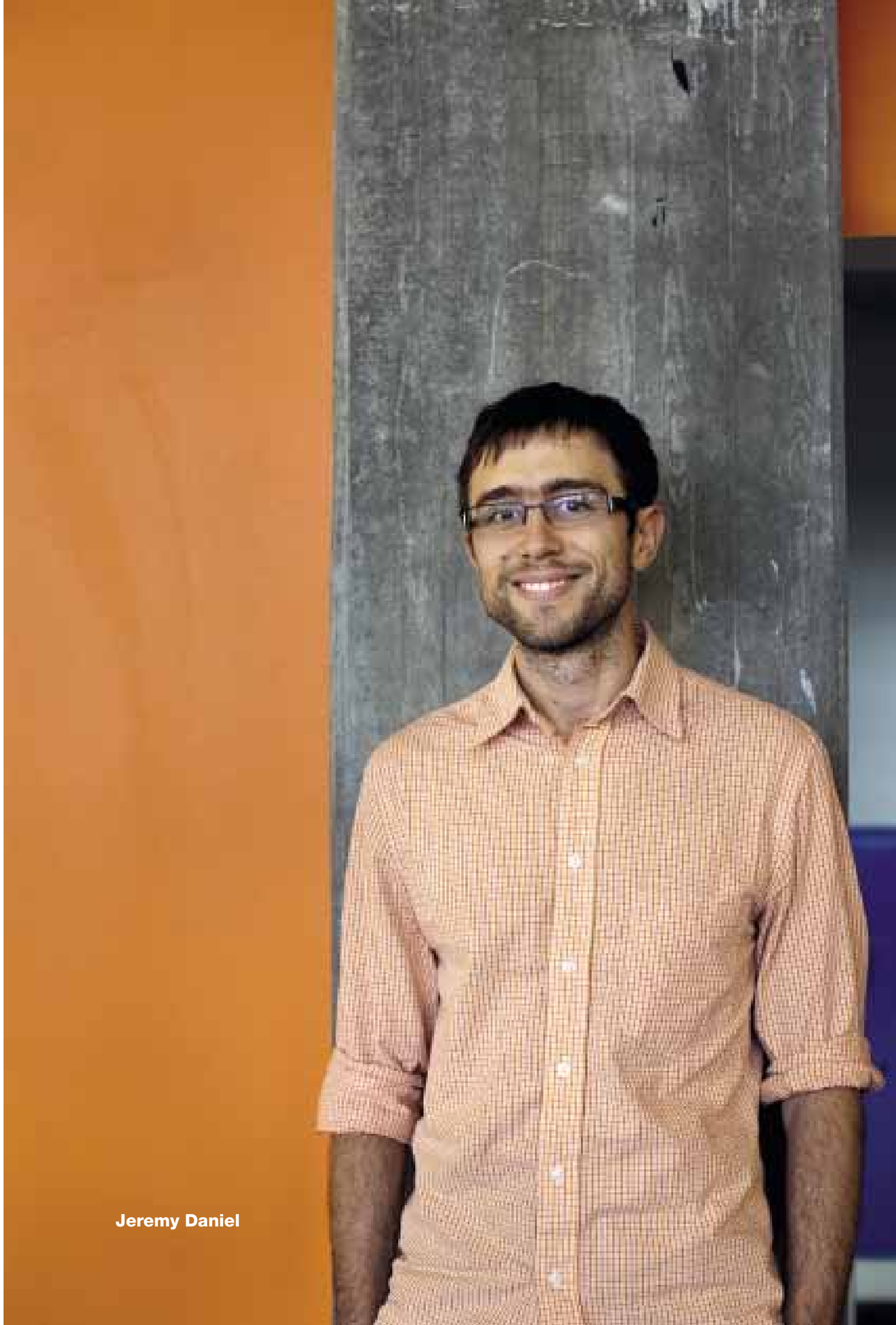
The Mitotic Mechanisms and Regulation research group started its activities at CPR in August 2011 headed by Assoc. Prof. Jakob Nilsson. Jakob is an established researcher within his focus area and brings a prestigious Junior Group Leader Fellowship from the Lundbeck Foundation to the Center, in addition to various other external grants. The group currently has four postdoctoral fellows, two PhD students and one Master student.

The research of the group aims at understanding how accurate chromosome segregation is achieved in human cells, since a failure in this process promotes the formation of cancer and is a hallmark of solid tumors. The chromosomes exist as tightly linked sister chromatids during mitosis and are separated by the mitotic spindle that pulls the sister chromatids to the opposite poles of the cell at anaphase. The microtubules of the mitotic spindle physically connect to chromatids at large protein structures referred to as kinetochores. Chromosome segregation is tightly regulated by the Spindle Assembly Checkpoint (SAC), which inhibits the separation of sister chromatids until all kinetochores have become properly attached to the mitotic spindle.

Through the combination of live cell imaging and in-house collaborations with the Facility for Protein Science and Technology (PST) and the Department of Proteomics, we aim at understanding:

- How does the kinetochore activate the SAC and how is microtubule binding sensed?
- How is the checkpoint silenced once the last kinetochore becomes attached?

We are also working closely with the Chemical Biology Unit in PST to make small molecule inhibitors against components of the proteolytic machinery responsible for exit from mitosis, which could prove useful in cancer therapy (see reference 1 on page 46).



Jeremy Daniel

Jakob Nilsson

Our ongoing collaboration with Tom Blundell's group at the Department of Biochemistry, University of Cambridge, UK, to study the structural aspects of the interaction between checkpoint proteins and kinetochore proteins, has recently led to the first structure of a checkpoint protein in complex with a kinetochore protein (see further details and reference on page 24).

Molecular Endocrinology

Group Leader, Prof. Amilcar Flores-Morales

The Molecular Endocrinology research group comprises three postdoctoral fellows, two PhD students and two research assistants.

The group aims to increase our understanding of the mechanisms driving prostate cancer progression. We are currently working on characterizing proteome changes associated with different stages of prostate cancer growth. In collaboration with the Department of Proteomics, we are using innovative protocols to analyze concentration changes of thousands of proteins in formalin-fixed paraffin-embedded clinical specimens. We utilize this information to identify novel pathways involved in carcinogenesis. The analysis of proteomic data obtained from clinical samples with associated long-term clinical follow-up also offers opportunities to identify biomarkers that can aid in the clinical management of the disease. In collaboration with clinical scientists at Umeå University, Sweden, we have initiated a project to identify prognostic biomarkers in prostate cancer through the analysis of prostatectomy samples with similar histological grade, but distinct, disease outcomes. A set of candidate biomarkers have been defined and further validation experiments are underway, analyzing tissue microarrays of several hundred tumor samples. We are also applying the methodology of proteomic profiling to study model systems and to characterize the signaling pathways regulating prostate cancer cell growth.

One of the main interests of the lab is to study the interplay between phosphorylation and ubiquitination, as mediated by the SOCS family of ubiquitin ligases, as well as the study of androgen receptor function in advanced, castration-resistant prostate cancer tumors. This year we have described the molecular basis for SOCS2 actions, detailing its ability to ubiquitylate the growth hormone (GH) receptor by assembling a Cullin/ring ubiquitin ligase complex (see reference 38 on page 48). We have also tested the involvement of SOCS2 in prostate carcinogenesis and metabolic control through the analysis of SOCS2 knock-out mice. SOCS2 is highly expressed in prostate tissue, where it opposes GH actions. Interestingly, metastatic prostate tumors express reduced levels of SOCS2. These findings may have clinical implications, as advanced tumors with diminished SOCS2 expression could consequently be sensitive to treatment with GH receptor antagonists.

In 2011, Prof. Amilcar Flores-Morales was awarded a research grant from the Danish Council for Independent Research, Medical Sciences to continue the studies on the SOCS2 knock-out mice. In addition, the work of the group is being supported by extensive collaborations both in-house and externally with clinical research groups in Europe and China.

Ubiquitin Signaling

Group Leader, Prof. Niels Mailand

In 2011 the Ubiquitin Signaling research group expanded further and now comprises 17 members, largely as a result of substantial external funding raised during 2010. Two international postdoctoral fellows and one PhD student were recruited. Moreover, Assoc. Prof. Mads Gyrd-Hansen joined the group together with a postdoctoral fellow and a PhD student specifically focusing on ubiquitin signaling in innate immunity.

We continued to expand and develop the portfolio of projects aiming to define novel roles for ubiquitin and ubiquitin-like modifiers in cellular responses to DNA damage. This was facilitated in particular by fruitful collaborations in-house (primarily with the Department of Proteomics and the Facility for Protein Science and Technology), as well as with a number of international partners. We are currently conducting detailed characterization of a range of ubiquitin-mediated and SUMO-mediated processes in the DNA damage response, providing new and exciting insight into how these modifiers function to safeguard genomic integrity.

At the time of writing, two papers with authors from the group have been published in 2011. Moreover, four manuscripts with the Ubiquitin Signaling Group members as lead authors are in submission or undergoing final revision for publication in high-impact journals. An additional four manuscripts arising from collaborative projects and involving authors from the group, are also in submission, and a number of additional projects are expected to be ready for submission in the first half of 2012. Collectively, these studies report new roles of ubiquitin and SUMO in genome stability maintenance, and identify novel factors involved in mediating such signaling processes. We thus expect a productive 2012 in terms of publication of high-impact papers from the group.

During the year, Simon Bekker-Jensen and Niels Mailand organized the 39th Annual Meeting of the Danish Society for Biochemistry and Molecular Biology on 'Frontiers in Cell Biology', and co-organized a PhD course on ubiquitin and ubiquitin-like modifier proteins.

Department of Disease Systems Biology

Research Director, Prof. Søren Brunak
Group Leader, Prof. Lars Juhl Jensen

The Department of Disease Systems Biology conducts multi-disciplinary basic research using bioinformatics and systems biology approaches in particular for analysis of large data sets of relevance to human health. The department is led by Prof. Søren Brunak and Prof. Lars Juhl Jensen and consists of one associate professor, three postdoctoral fellows, nine PhD students and one Master student.

Department Update
The Department of Disease Systems Biology consists of two independent research groups, each of which has established its own line of research, but also carries out joint projects. The work of the department is largely data driven and taps into the readouts from experiments within personalized genomics and proteomics, which are currently overwhelming the biological and medical research areas with computer-accessible data. The goal is to improve our understanding of human health, disease mechanisms, pharmaceutical targets and, in the case of basic biology, systems-level properties, for example related to signaling, protein modification and localization.

The life sciences are scaling up and producing huge amounts of data and new literature at an amazing pace. To help researchers get an overview of the scientific literature and associated data, we are involved in the development of numerous community resources for data and text mining, such as NetPhorest, NetworKIN, Reflect, STRING and STITCH. These are developed in close collaboration with research groups at the European Molecular Biology Laboratory, Institute of Cancer Research (UK), University of Zurich (Switzerland), TU Dresden (Germany) and the Technical University of Denmark. In 2011, the group published another resource – DistiLD – that links disease information from genome-wide association data to protein coding genes (see reference 13 on page 46).

In 2011 the department has further developed an in-house platform for efficient mining of biomedical texts. This enables us to efficiently map terms in free text to structured vocabularies, which we are currently using *(i)* to derive interaction networks for proteins from the scientific literature, *(ii)* to link genes and proteins to the diseases in which they are involved and *(iii)* to extract information on diagnoses, medication and adverse drug events from electronic patient records. Additionally this infrastructure is being used to provide several external collaborators with text-mining support. Our first publication on electronic record text mining and the link to network biology was published in 2011 (see further details and reference on page 20). In a new project we have initiated a large scale analysis of the entire Danish National Discharge Registry, with a similar aim of validating disease co-occurrences and discovering disease genes shared between diseases.

The department has continued its close collaboration with the Department of Proteomics on analysis of, in particular, post-translational modifications identified by mass spectrometry. In 2011 this resulted in joint publications on lysine acetylation and ubiquitylation as well as two publications with external collaborators on paleoproteomics (see further details and references on page 22). We have recently hired a joint PhD student, working with both departments, to further strengthen the collaboration. We have also contributed to work driven by external collaborators on the analysis of the protein complement resulting from the first genome sequence of an Australian aborigine. This publication appeared on the cover of Science in 2011 (see reference 18 on page 47).

Awards
Søren Brunak was made fellow of the International Society of Computational Biology in 2011. In November 2011 Søren Brunak was appointed as chairman of the interim board of the emerging European infrastructure for bioinformatics, ELIXIR, under the ESFRI program.

Department of Proteomics

Research Director, Prof. Matthias Mann
Group Leader, Prof. Jesper Velgaard Olsen, Mass Spectrometry for Quantitative Proteomics
Group Leader, Assoc. Prof. Chuna Ram Choudhary, Proteomics and Cell Signaling
Group Leader, Assoc. Prof. Michael Lund Nielsen, Proteomics Technology Development and Application

The Department of Proteomics consists of three independent research groups, each of which has established its own line of research. However, the unifying theme in the department is unbiased and global analysis of post-translational protein modifications in cell signaling networks and the development of proteomics technologies to enable this. A growing area of research for the department is to analyze signaling crosstalk on a proteome-wide scale. We are also currently establishing a technology platform for high-throughput and parallel protein-protein interaction screens.

Department Update
Since the inauguration of CPR, the Department of Proteomics has grown into an international research environment, currently consisting of more than 25 scientists (including 14 postdoctoral fellows and six PhD students) with nine different nationalities covering four continents. During 2011, the department established a large number of collaborations with both local and international research groups, including Prof. Eske Willerslev and Prof. Steve Jackson (see further details and references on page 22). Moreover, the proteomics accessibility scheme, which allows external research groups to collaborate with our department on research projects of mutual interest, was fully implemented during 2011. So far this implementation has been a success, with 2/3 of the proposed collaborations being established with leading research groups from the Medicon Valley area.

A main objective for the department is continuous technological development of the integrated proteomics platform. During the year the mass spectrometric instrument park was upgraded to six of the latest generation of orbitrap mass analyzers, the Q-Exactive mass spectrometers. In addition, the department has purchased two ultra-high performance liquid-chromatography (UHLPC) systems for improved chromatography performance. With the purchase of these instruments we have extended our sequencing capacity, and furthermore ensured that the department retains its competitive technological edge.

During 2011 the department co-authored and published more than 15 papers in international peer-reviewed journals such as Science and Science Signaling (see list of publications on page 46-49 reference 2, 4, 6, 16, 17, 27, 29, 31, 33, 34, 36, 39, 42, 44, 47, 48 and 51).

Grants and Awards
Group Leader, Prof. Jesper Velgaard Olsen, was awarded a prestigious Sapere Aude: DFF Starting Grant from the Danish Council for Independent Research - Medical Sciences. He received more than DKK 8 million (EUR 1.1 million) for his project entitled: 'Phosphoproteomics Tracing of Cancer Therapeutic Drug Effects in Signaling Networks from Tissues and Organs'. In addition, Jesper was awarded DKK 250,000 (EUR 34,400) from the Faculty of Health Sciences.

Brian Tate Weinert, postdoctoral fellow, was awarded the best oral presentation prize at the European Proteomics Association (EuPA) meeting organized in conjunction with the Human Proteome Organization (HUPO) World Congress 2011 in Geneva, Switzerland.

Research Director, Prof. Matthias Mann, Department of Proteomics, CPR and the Max Planck Institute for Biochemistry was awarded the 2012 Gottfried Wilhelm Leibniz Prize for his development of methods for analysis of proteins using mass spectrometry. The Leibniz Prize is the most important and prestigious award to individual scientists in Germany, with a prize money of EUR 2.5 million.

Facility for Protein Science and Technology

The main focus for the Facility for Protein Science and Technology (PST) is to develop new methods and to implement and optimize existing state-of-the-art methodologies and technologies in the areas of protein production and chemical biology as well as protein-ligand and protein-protein interactions. PST now consists of three organizational parts, the Protein Production Unit (PPU), the Chemical Biology Unit (CBU) and the newly established Protein Function and Interaction Unit (PFI, from November 2011). Six publications were published by PST staff during 2011 (see list of publications on page 46-49, reference 12, 14, 30, 37, 40 and 46).

Since October 2011 the chief scientist (Michael Sundström) has left PST, and it is *ad interim* jointly being managed by the three unit heads (Jens Berthelsen, Bjørn Voldborg and Mats Wikström).

At the end of 2011, PST employed 42 staff and affiliated members, with more than half of the staff dedicated to individual research projects focusing on biological systems and methodology development.

Protein Production Unit

Unit Head, Bjørn Voldborg

The development efforts in the Protein Production Unit (PPU) have mainly focused on the implementation of a new high-throughput (HT) eukaryotic expression platform based on HEK293-EBNA cells, to complement the *E. coli* platform previously established. Using the existing *E. coli* production platform, PPU generated more than 4,800 DNA constructs in 2011, representing 390 different protein targets. Of these, more than 180 produced soluble protein in small-scale expression screening, resulting in 234 large-scale expressed and purified proteins available to our collaborators. Some of these proteins have been produced for the Department of Proteomics, (BRCT and FHA domain containing proteins) and for the Molecular Neuropharmacology Group (Dr. Gether) at the Faculty for Health Sciences, University of Copenhagen (PDZ domain containing proteins).

As a part of the Affinomics project (focused on the generation of high quality and renewable protein affinity binders, such as monoclonal antibodies) under the European Framework 7 program, PPU has produced and shipped more than 250 protein samples to several world-leading binder-producing laboratories all over Europe.

For maximal scientific leverage from the proteins produced, information on most of the purified proteins is also made available to the scientific community through the CPR Protein Resource webpage, www.cpr.ku.dk/protein_resource/, and can be requested from PPU on a collaborative basis.

To meet the needs of our collaborators, PPU has constructed more than 40 new expression vectors, enabling the fusion of a large number of commonly used tags and combinations thereof to expressed proteins, facilitating subsequent experiments, including purification, pull-downs and detection.

To further develop the eukaryotic expression platforms, our present focus is on improving the HEK293-EBNA-based HT expression platform, with particular attention on expression and purification yields. We have so far been able to purify several proteins (such as the kinase BLK) for the Chemical Biology Unit.

In parallel to running and developing the pipeline expression systems described above, PPU is also working on protein-family-specific projects, focused on membrane proteins, (such as TRP channels and GPCRs). One postdoctoral fellow is currently working on the expression, purification and characterization of TRP channels, while another is expressing, purifying and characterizing several GPCR membrane proteins as part of the collaboration with the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen (Prof. Thue Schwartz). During 2011 PPU hosted a PhD course, a number of visiting scientists, and a two-day international workshop focused on protein production.

Chemical Biology Unit

Unit Head, Assoc. Prof. Jens Berthelsen

The overall goals for the Chemical Biology Unit (CBU) are to identify and characterize protein-ligand interactions for natural and chemical modulators of protein function, with the scope of being able to study protein function in a biological context, as a starting point for translational research. We also focus on how chemical modifications of therapeutically relevant proteins can influence their properties.

During the past year, activities within the unit were focused on utilizing our technological platforms to investigate selected protein targets and areas, often in collaboration with external research groups. We have also been finalizing the unit organizational outline with additional recruitments. At the end of 2011, CBU consisted of 18 staff and affiliated members.

The group integrates expertise in chemoinformatics, bioassays and biophysics, and applies these to specific scientific problems.

The Chemoinformatics Team is now jointly run with the Novo Nordisk Foundation Center for Basic Metabolic Research (CBMR) at the University of Copenhagen. This joint operation builds on the knowledge within CBU chemoinformatics, integrated with the know-how at CBMR in pharmacological property analysis of compounds.

The Bioassay Team has worked on implementing a high degree of automation for screening as well as for compound plate handling. We are now set up to run enzyme activity-based screens, based on micro fluidic capillary electrophoresis as well as fluorescence readout, and ligand-binding assays based on differential scanning fluorimetry (DSF). All methods are running in a 384-well format.

The Biophysics Team harbors a unique, state-of-the-art instrumentation park, dedicated to the biophysical characterization of protein-protein, and protein-ligand interactions. The team has excellent know-how regarding methodologies such as analytical ultra centrifugation (AUC), isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), surface plasmon resonance (SPR) using a Biacore T200, dynamic light-scattering (DLS) and intrinsic fluorescence. The group has experienced a large number of requests for collaboration and analysis, from both in-house and many external groups.

In addition to the unit's core activities, three postdoctoral fellows, and six PhD and Master students are associated with CBU, pursuing specific projects. These include protein modifications and property profiling (PMPP), kinase inhibitor profiling, ADAM proteases, ubiquitin signaling and methyl transferases. In addition, CBU has had a number of visiting scientists. This year we also held a practical PhD course in Chemical Biology for 11 students. Our first Master student graduated during 2011 after a one year Master thesis in PST.

During the past year, CBU has established and continued various strategic collaborations, including a number of in-house projects. External collaborators include Dr. Ming-Wei Wang (National Center for Drug Screening Shanghai, China), for the synthesis of novel chemical probes; Prof. Stefan Knapp (SGC Oxford, UK) on certain protein kinases inhibitor profiling; Prof. Olli Silvennoinen (Tampere, Finland) on the regulation of JAKs; Prof. Niels Ødum (University of Copenhagen) on lymphoma-related kinases and Prof. Knud Jensen, Prof. Marco van de Weert, Prof. Hanne Mørck-Nielsen (University of Copenhagen) and Dr. Lars Fogh Iversen (Novo Nordisk A/S) in our protein modifications and property profiling project.

Protein Function and Interactions Unit

Unit Head, Assoc. Prof. Mats Wikström

With the introduction of the Protein Function and Interactions Unit (PFI), we have strengthened our research in the area of biophysical and structural studies, which are important components for most protein-focused research projects. Research within the unit focuses on understanding molecular recognition through biophysical and structural studies of protein-protein interactions applied to systems of biomedical importance. At the time of writing the unit consists of five members including one PhD student and two postdoctoral fellows. The unit is currently active in three collaborative research areas involving in-depth studies of the components and complexes of the interacting proteins.

The biophysical studies are carried out using the existing infrastructure at PST, while nuclear magnetic resonance (NMR) experiments are performed at Biophysical Chemistry, Lund University, and at the Swedish NMR Centre in Gothenburg, Sweden. X-ray crystallographic studies will be performed at the MAX-lab synchrotron station, Lund University. Although data collection will be performed using external facilities, data analysis and refinement of the NMR and X-ray data will be done at PST in Copenhagen.

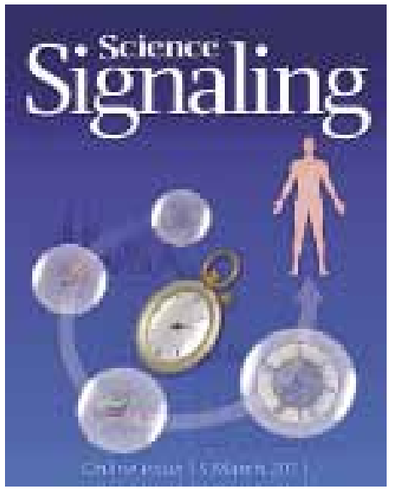
PFI's well-established in-house collaboration with the Ubiquitin Signaling research group has recently led to the identification of a novel SUMO-binding motif. In addition, several external strategic collaborations have been initiated, including a PhD project related to IGF signaling with Dr. Ming-Wei Wang (National Center for Drug Screening Shanghai, China) and an interactome study on a family of novel secreted proteins from the human pathogen *Streptococcus pyogenes* in collaboration with Assoc. Prof. Johan Malmström (Lund University, Sweden).


Steril filtrerat
Vatten

Scientific Case Stories

System-wide Analysis of Human Stem Cell Differentiation

The Department of Proteomics (Jesper Velgaard Olsen and Matthias Mann), in collaboration with the group of Assoc. Prof. Dr. Blagoy Blagoev at the Center for Experimental BioInformatics, University of Southern Denmark, and other international partners, published a paper in Science Signaling in March 2011. The article was titled 'System-wide Temporal Characterization of the Proteome and Phosphoproteome of Human Embryonic Stem Cell Differentiation' and was also featured on the cover page of the journal.



Rigbolt KT *et al*., Science Signaling. 2011 March 15;4(164):rs3.

Understanding the signaling events that control stem cell pluripotency and self-renewal, as well as those governing differentiation, should improve our ability to develop stem cell-based therapies. We are therefore setting out to decipher the dynamics of the stem cell phosphoproteome. In the current study we performed global quantitative proteomic and phosphoproteomic analysis of human embryonic stem cells at five time points over 24 hours of non-directed (lineage-independent) differentiation, initiated by two different paradigms. We identified a common core phosphoproteome associated with both differentiation protocols, discovered several temporal patterns of phosphorylation, and made predictions about changes in the activities of kinases during the differentiation period. DNA methyltransferases (DNMTs) exhibited dynamic changes in phosphorylation status that may influence their interaction with a promoter-bound protein complex, suggesting that the phosphorylation state of DNMTs may govern their recruitment to, and thus silencing of, target genes such as those that promote pluripotency during differentiation.

Identifying Correlations in Electronic Patient Records

CPR researchers from the Department of Disease Systems Biology (Prof. Søren Brunak, Prof. Lars Juhl Jensen and PhD student Peter Bjødstrup Jensen) contributed to a study demonstrating how text mining of electronic health records can be used to create medical term profiles of patients, which can be used both to identify co-occurrence of diseases and to cluster patients into groups with highly similar clinical features. The study, carried out in Denmark by a multi-disciplinary group of bioinformaticians, systems biologists and clinicians led by Prof. Søren Brunak, was published in the open-access journal PLoS Computational Biology on August 25, 2011.

Health records contain detailed phenotypic information on the clinical profile of each individual patient; however, a large part of the clinical features are described in free text produced by hospital staff, often covering many years of hospitalization. Using our text-mining approach on the free text in the records, we identified roughly ten-times as many medical terms characterizing each patient as were manually included by the hospital staff. Worldwide, the manually inserted medical terms in medical records are heavily biased by local practice and billing purposes. Using our method we obtained a much more fine-grained clinical characterization of each patient, which ultimately may also be very valuable for choosing personalized treatment regimes.

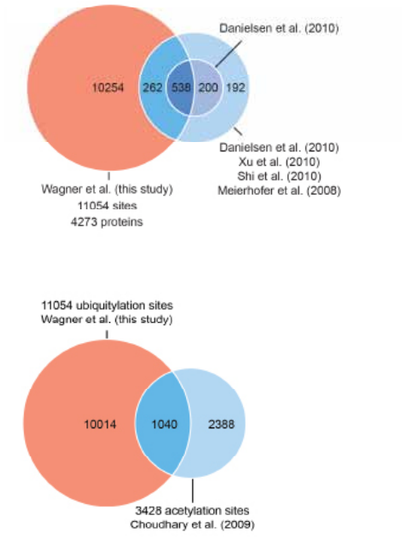
A network depicting patients' health problems (colored dots) reveals overlapping conditions, including known connections such as diabetes (light orange, numbered 26 at top) and hypertension (dark green, numbered 72, just to the right). Roque FS, *et al*. PLoS Comput Biol. 2011, 7(8): e1002141.

The team used the International Classification of Disease (ICD) terminology, a controlled vocabulary maintained by WHO, as the basis for the analysis. Because ICD has been translated word-by-word between languages, the procedure is in principle language independent and opens up new exciting possibilities of e.g. combining cohorts from different countries.

The research group identified a large number of diseases and symptoms which co-occur much more than expected when compared to the individual frequencies of the diseases. The group subsequently mapped these correlations at genetic level by investigating gene overlaps in protein interaction networks already linked to the individual diseases with the aim to discover a possible genetic cause behind the disease correlations observed, thus directly interfacing electronic patient-record data to the DNA sequencing of human individuals.

Large-scale Analysis of Ubiquitylation Sites

Modification of proteins by ubiquitin can serve as a 'kiss of death' to target proteins for degradation via the proteasome. It is now becoming clear that ubiquitin plays many other important functions in cells. However, precise mapping of modification sites and their quantification has remained a major challenge.



An overview of ubiquitylome and its overlap with acetylome. The upper panel shows the number of ubiquitylation sites identified in the current study and their overlap with previously known sites. The lower panel shows the substantial overlap between ubiquitylation sites with known acetylation sites in human cells. This research was originally published in Molecular & Cellular Proteomics. Wagner SA, *et al*. A proteome-wide, quantitative survey of *in vivo* ubiquitylation sites reveals widespread regulatory roles. Mol Cell Proteomics. 2011 Oct;10(10):M111.013284. © the American Society for Biochemistry and Molecular Biology.

The Department of Proteomics (Sebastian Wagner, Petra Beli, Brian Weinert, Michael Lund Nielsen, Matthias Mann and Chuna Ram Choudhary) recently published the largest ubiquitylation dataset on human cells to date. In this study, for the first time, researchers were able to quantify changes in thousands of endogenous ubiquitylation sites in cells treated with a drug that inhibits ubiquitin-mediated protein degradation via the proteasome.

Wagner *et al*. precisely mapped more than 11,000 endogenous putative ubiquitylation sites, of which over 90% represent novel, previously non-reported sites. Previously, researchers from the Center used another affinity-purification-based approach to catalogue the, until then, largest number of ubiquitylation sites (Danielsen *et al*., MCP, 2010). By further improving the proteomics methods, the current study significantly expands the number of currently known human ubiquitylation sites and will serve as a valuable resource for future functional characterization of many proteins. The researchers show that ubiquitylation target proteins are involved in all major cellular functions and report that nearly half of all sites were shown to have non-proteasomal functions. The study concludes that the regulatory scope of ubiquitylation is comparable to other post translational modifications such as phosphorylation and acetylation. The novel approach described in this paper is generic, and opens new avenues for global quantification of ubiquitylation changes in cells and tissues.

External Collaborations

First Ancient Proteome Revealed

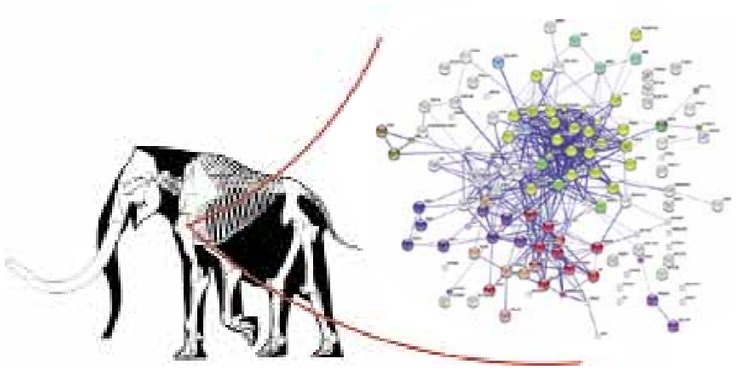
The groups of Lars Juhl Jensen and Jesper Velgaard Olsen (Department of Disease Systems Biology and Department of Proteomics) together with Eske Willerslev and colleagues at the Centre of Excellence in GeoGenetics at the Natural History Museum of Denmark and international co-workers used high-sensitivity, high-resolution tandem mass spectrometry to sequence proteins extracted from a 43,000 year-old woolly mammoth bone that had been preserved in Siberian permafrost. For the first time, 126 unique ancient proteins, mostly low-abundance extracellular matrix and plasma proteins, were confidently identified by unambiguous molecular evidence. Among these prehistoric proteins, the best characterized was the carrier protein serum albumin, presenting two single amino-acid substitutions compared to extant African *(Loxodonta africana)* and Indian *(Elephas maximus)* elephants. Strong evidence was observed of amino-acid modifications due to post-mortem hydrolytic and oxidative damage. A consistent subset of this permafrost bone proteome was also identified in more recent Columbian mammoth *(Mammuthus columbi)* samples from temperate latitudes, extending the potential of the approach beyond subpolar environments. Mass spectrometry-based ancient protein sequencing offers new perspectives for future molecular phylogenetic inference and physiological studies on samples not amenable to ancient DNA investigation. This approach therefore represents a further step in the ongoing integration of different high-throughput technologies for identification of ancient biomolecules, inaugurating the field of paleoproteomics. The paper has been highlighted in Nature News and in the American Chemical Society's Chemical and Engineering News.

High-resolution Tandem Mass Spectrometry Identifies Origin of Bone Projectile Point

In collaboration with Eske Willerslev, Centre of Excellence in GeoGenetics at the Natural History Museum of Denmark, scientists at the Department of Disease Systems Biology and Department of Proteomics used high-resolution tandem mass spectrometry-based protein sequencing to determine the origin of a bone projectile point found in mastodon remains at the Manis site in Washington state, US. The data adds to the evidence that people were hunting mastodons at least two millennia earlier than Clovis and sheds new light on the prehistoric human presence in North America. Citation: Waters MR, *et al*. Science. 2011 Oct 21;334(6054):351-3.

My team at the Centre of Excellence in GeoGenetics has in the past year collaborated with the groups led by Prof. Jesper Velgaard Olsen and Prof. Lars Juhl Jensen at The Novo Nordisk Foundation Center for Protein Research. We have benefitted from each other's knowledge on ancient biomolecules and protein sequencing. The collaboration has so far resulted in two publications; one in Science on early peopling of the Americas and the other in Journal of Proteomic Research on woolly mammoth proteomics. The collaboration is a perfect example of how collaborations across the interface of different disciplines can result in something new and exciting.

– Eske Willerslev
Prof. Director, Centre of Excellence in GeoGenetics,
University of Copenhagen, Denmark



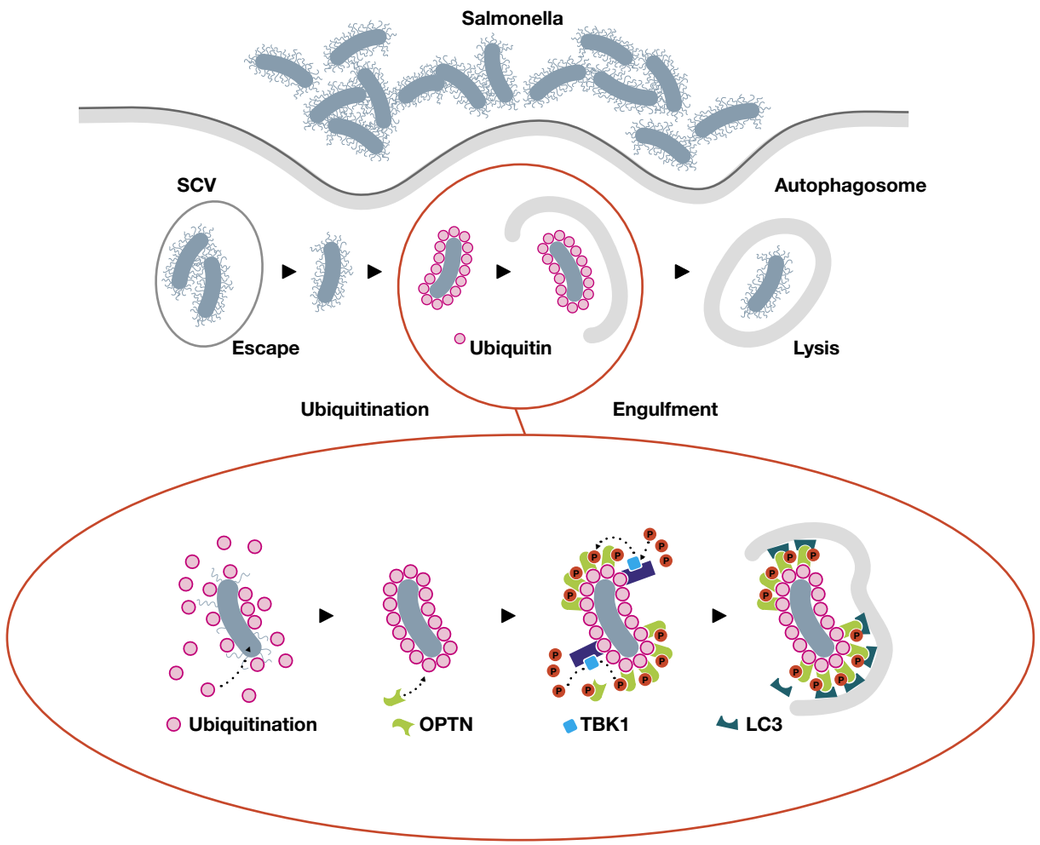
Reprinted with permission from Cappellini E, *et al*. Proteomic analysis of a Pleistocene mammoth femur reveals more than one hundred ancient bone proteins. J. Proteome Res. Epub ahead of print: 2011 Dec 14. Copyright 2011 American Chemical Society.

New Defense Mechanism against *Salmonella* Elucidated

In collaboration with a team led by Prof. Ivan Dikic, Institute of Biochemistry II, the Goethe University Frankfurt, Germany, the Department of Proteomics applied a state-of-the-art mass spectrometry technique to elucidate the molecular mechanisms that contain the spread of the pathogenic bacteria *Salmonella* in human cells. These findings identify phosphorylation of the autophagy receptor Optineurin as a key mechanism that restricts the spread of *Salmonella* by eradicating the pathogen through selective autophagy.

It was a great pleasure to engage in collaboration with Chuna Ram Choudhary and Sebastian Wagner from CPR. We have analyzed post-translational modifications critical for the removal of the intracellular pathogen *Salmonella typhimurium* via the autophagy pathway. The key to our success was open and intensive communication in order to use the high-end proteomic approaches to address important biological questions. The professional attitude of Sebastian and Chuna was exceptional and we really enjoyed our collaborative work.

– Ivan Dikic
Director of the Institute of Biochemistry,
Goethe University, Frankfurt
Scientific Director of the FMLS/Frankfurt Institute for Molecular Life Sciences, Germany



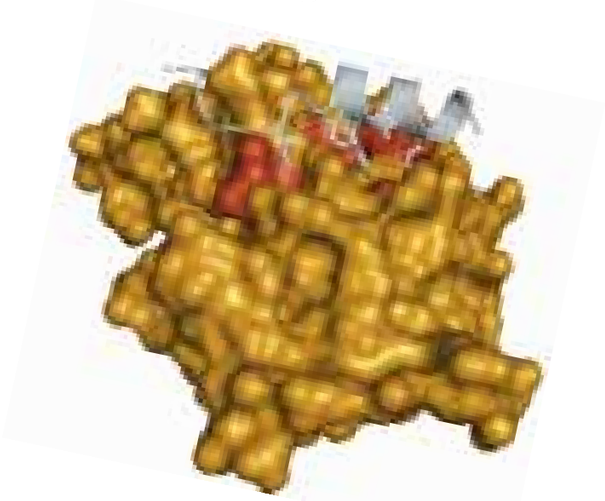
Optineurin (OPTN) functions as a novel autophagic receptor for eliminating cytosolic *Salmonella*. Phosphorylation of OPTN by TBK1 kinase enhances its binding to LC3 and induces selective autophagy of the cytosolic bacteria. Adapted from Galluzzi L, *et al*. EMBO J. 2011 Jul 22;30(16):3213-4 and Wild P, *et al*. Science. 2011 Jul 8;333(6039):228-33.

Getting Checkpoint Proteins to the Kinetochore

Chromosome segregation is achieved by microtubules of the mitotic spindle binding to structures on chromosomes referred to as kinetochores. Maintenance of genomic stability relies on the spindle assembly checkpoint (SAC), which ensures accurate chromosome segregation by triggering anaphase delay in response to kinetochores incorrectly attached, or not attached, to the mitotic spindle.

Ongoing collaborations with Tom Blundell's group at the Department of Biochemistry, University of Cambridge, UK, and Jakob Nilsson and colleagues from Mitotic Mechanisms and Regulation, Department of Disease Biology, are focusing on the structural aspects of the interaction between checkpoint proteins and kinetochore proteins. This recently led to the elucidation of the structure of the checkpoint protein BubR1 in complex with the kinetochore protein KNL1. The mode of interaction was unanticipated and showed that KNL1 and BubR1 interact through complementary hydrophobic interfaces. Using RNAi and rescue experiments we could show that residues predicted from the structure to be important for the interaction are indeed critical for a functional checkpoint in cells.

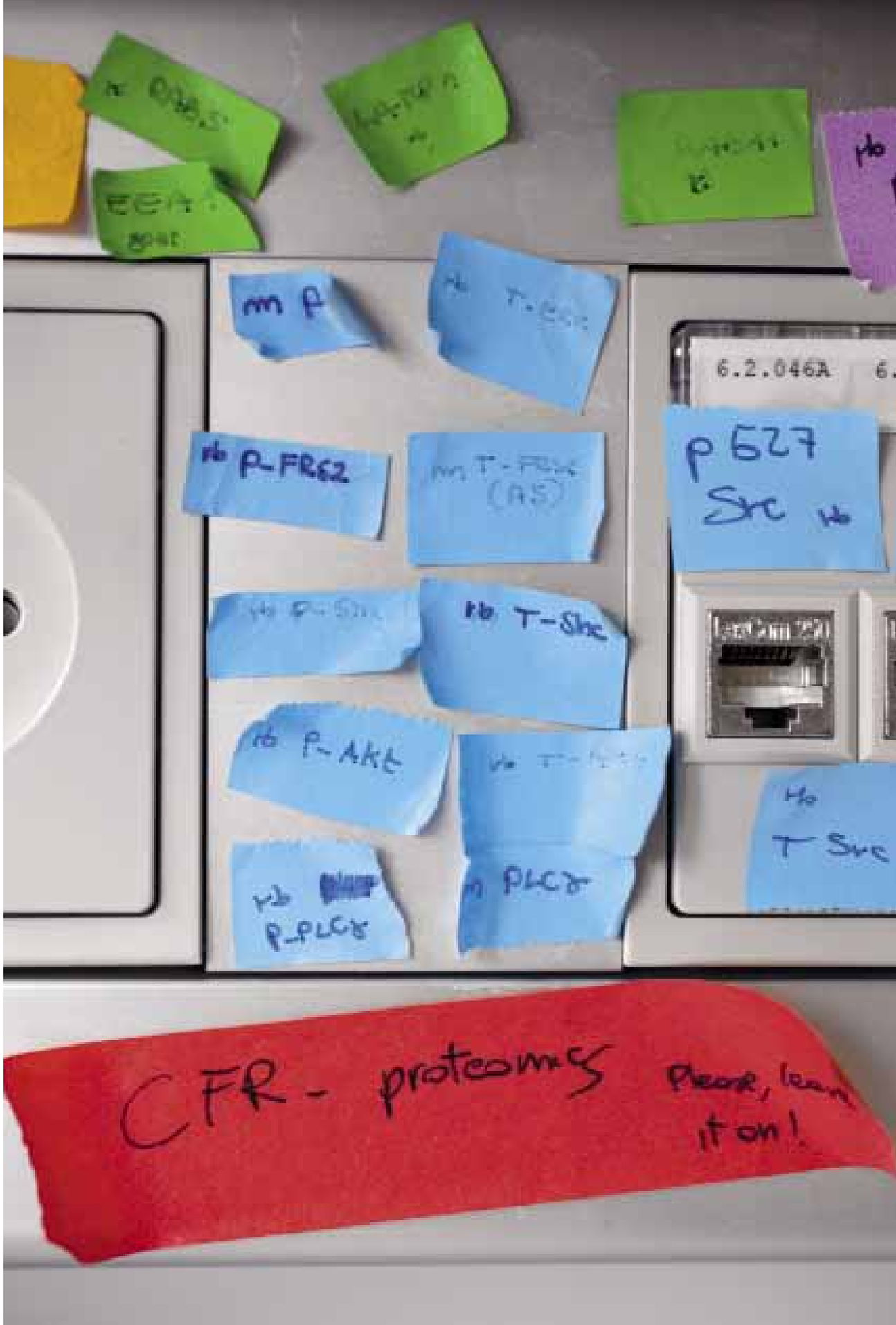
This work is the first structure of a checkpoint protein in complex with a kinetochore protein and underscores the importance of recruitment of checkpoint proteins to the kinetochore for a functional checkpoint. The structure furthermore paves the way for designing and identifying small molecules able to inactivate the SAC which could be useful for cancer therapy.



Insight into the interaction between the kinetochore protein KNL1 and the checkpoint protein BubR1. Surface representation of BubR1 in orange with red indicating residues making contact to KNL1 (in grey). The critical residues in KNL1 for the interaction with BubR1 are represented by sticks. Bolanos-Garcia VM, *et al.* Structure. 2011 Nov 9;19(11):1691-700.

We have benefitted from the expertise of Prof. Jakob Nilsson and his group on the cell biology of mitosis. The commitment and professionalism of Jakob and Tiziana have been instrumental in proving that recruitment of checkpoint proteins to the kinetochore is essential for a functional checkpoint. Furthermore, the first structure of a checkpoint protein in complex with a kinetochore protein that we reported recently paves the way for designing and identifying small molecules able to inactivate the SAC, and this could be useful for cancer therapy. We are very excited about the prospect of continuing our collaboration with Prof. Jakob Nilsson and his group to explore these and other opportunities.

– Tom Blundell
Prof. Group Leader
Crystallography and Bioinformatics Group
Department of Biochemistry
University of Cambridge, UK



Training and Education

Contributing to the training of young scientists is a central mission of the Center and during the year we organized four PhD courses for the Faculty of Health Sciences. One of the new initiatives was the laboratory-based course: ‘Understanding Protein Function through Chemical Biology’ provided by the Chemical Biology Unit, Facility for Protein Science and Technology (PST). Eleven students had the opportunity to get hands-on training in technologies and methods in protein structure assessment, protein-ligand structural modeling, binding and functional screening assays, and biophysical assessment of interaction characteristics. The focus of the course was on how to use chemical compounds (bioprobes) to investigate the function of proteins and protein-protein interactions. Identification of bioprobes is emerging as an important tool for the analysis of protein function in a biological context and for identification and validation of novel therapeutic targets. Another laboratory-based PhD course: ‘High-throughput Cloning and Protein Production’, was offered by the Protein Production Unit, PST, providing hands-on experience in cloning, expression and production of a large number of proteins in *E. coli* cells.

Two theoretical PhD courses were also offered by CPR during 2011 combining lectures, journal clubs and student presentations. Both courses were highly appreciated for numerous talks by internationally recognized experts within respective fields. ‘Mass spectrometry-based Proteomics and its Applications in Biology’ was organized by the Department of Proteomics, focusing on the latest developments within the field of proteomics. Local as well as international experts teaching on the course included Jens Andersen, Frank Kjeldsen and Blagoy Blagoev (University of Southern Denmark), Marcus Krüger (Max-Planck Institute for Heart and Lung Research, Germany) Jürgen Cox and Henrik Daub (Max Planck Institute for Biochemistry, Germany), Matthias Selbach (Max Delbrück Center for Molecular Medicine, Germany), Michiel Vermeulen (Molecular Cancer Research, University of Utrecht, The Netherlands) and Boris Macek (Proteome Center Tübingen, Germany).

The Ubiquitin Signaling and the Mitotic Mechanisms and Regulation research groups, Department of Disease Biology, co-organized ‘Ubiquitin and Ubiquitin-like Modifiers’ covering structural and functional aspects as well as methods used to study ubiquitin and ubiquitin-like modifiers. The course featured excellent presentations by David Komander, (MRC Laboratory of Molecular Biology, Cambridge, UK), Alfred Vertegaal (Leiden University Medical Center, The Netherlands), Ronald T. Hay (Wellcome Trust Centre for Gene Regulation and Expression, Scotland), David McEwan (Institute for Biochemistry II, University of Frankfurt Medical School, Germany) and Rasmus Hartmann-Petersen (Department of Biology, University of Copenhagen, Denmark).

Four courses will be offered at CPR in 2012, aiming to provide excellent training opportunities for young researchers in and around Copenhagen and Denmark. The Center currently has 28 PhD students and 36 postdoctoral fellows receiving their scientific training in our different research groups. We also contributed to undergraduate teaching by means of supervising six Master students during the year.

Mads Grønborg, Jannie Rendtlew Danielsen and Erik Vernet

Successful Alumni

It has been exciting to see some of the first postdoctoral fellows we employed move on to prominent positions both within academia and industry. Part of our mission is to grow and develop some of the best research talent for further careers within protein science. Training at CPR offers dynamic and cutting edge research opportunities and ensures an excellent knowledge base and know-how for our postdoctoral fellows and other staff at the Center. We feel confident that the Center will make an impact on Danish and international research environments as we start to see the effects of successfully trained staff having left CPR to pursue their future research careers. Erik Vernet, former Novo Nordisk R&D – Science, Talent, Attraction and Recruitment (STAR) program postdoctoral fellow in the Facility for Protein Science and Technology is now a research scientist at Novo Nordisk A/S and Mads Grønborg, also a former STAR postdoctoral fellow, who trained at the Department of Proteomics, is now a Senior Scientist at the Hagedorn Research Institute, Novo Nordisk. In addition, postdoctoral fellow Jannie Rendtlew Danielsen from the Ubiquitin Signaling research group, Department of Disease Biology, is currently gaining new expertise as a visiting scientist with Prof. Yungui Yang, Genome Stability Group, Beijing Institute of Genomics, Chinese Academy of Science, as part of her Sapere Aude: DFF postdoctoral fellowship (Danish Council for Independent Research). This experience will allow Jannie to further develop and strengthen her independent scientific potential, eventually paving the way for yet another successful sortie from CPR.

After eight years of working abroad at John Hopkins University in Baltimore and at the Max Planck Institute in Göttingen, I had an opportunity to work at the Department of Proteomics at CPR from 2010 to 2011. I worked in the group headed by Jesper Velgaard Olsen, which focuses on developing and using quantitative proteomics to study perturbations in cell signaling and on identifying post-translational modifications (in particular phosphorylation) on a global scale. My research at CPR focused on identifying novel markers involved in inflammation.

The high level of expertise and interdisciplinary groups at CPR have made it a very attractive and inspirational work place for me. This combination is essential, since it allows for the easy and fast collaborative efforts needed in high impact research. The Center's close collaboration with Novo Nordisk was also attractive to me, since it allowed me to do more applied science. I am now very pleased to be taking up a position at the R&D department at Hagedorn Research Institute, Novo Nordisk – a possibility strongly facilitated by my stay at CPR.

– Mads Grønborg

I worked as a postdoc at the Facility for Protein Science and Technology at CPR between 2009 and 2011. Here, I contributed to the implementation and further development of high-throughput protein expression system for *E.coli*, and used this to produce >25 biomedically relevant human proteins.

I believe that the dynamic mixture of different research areas and scientific competences found at the Center is one of its core strengths – there is always someone to ask and discuss with! I am also very grateful for the substantial funding available for my project, both indirectly (through the Novo Nordisk Foundation and the University of Copenhagen), and directly (through a personal STAR fellowship grant from Novo Nordisk A/S), which allowed me to work with state-of-the-art technologies throughout my project. This was especially important for the protein characterization studies, with direct access to advanced and expensive technologies such as SPR, DSC, ITC and AUC.

Finishing my postdoc, I have taken up a position with Novo Nordisk A/S, where I use many of the skills developed at CPR on a daily basis. Thus, I can testify that working at CPR had a major impact on my career path.

– Erik Vernet

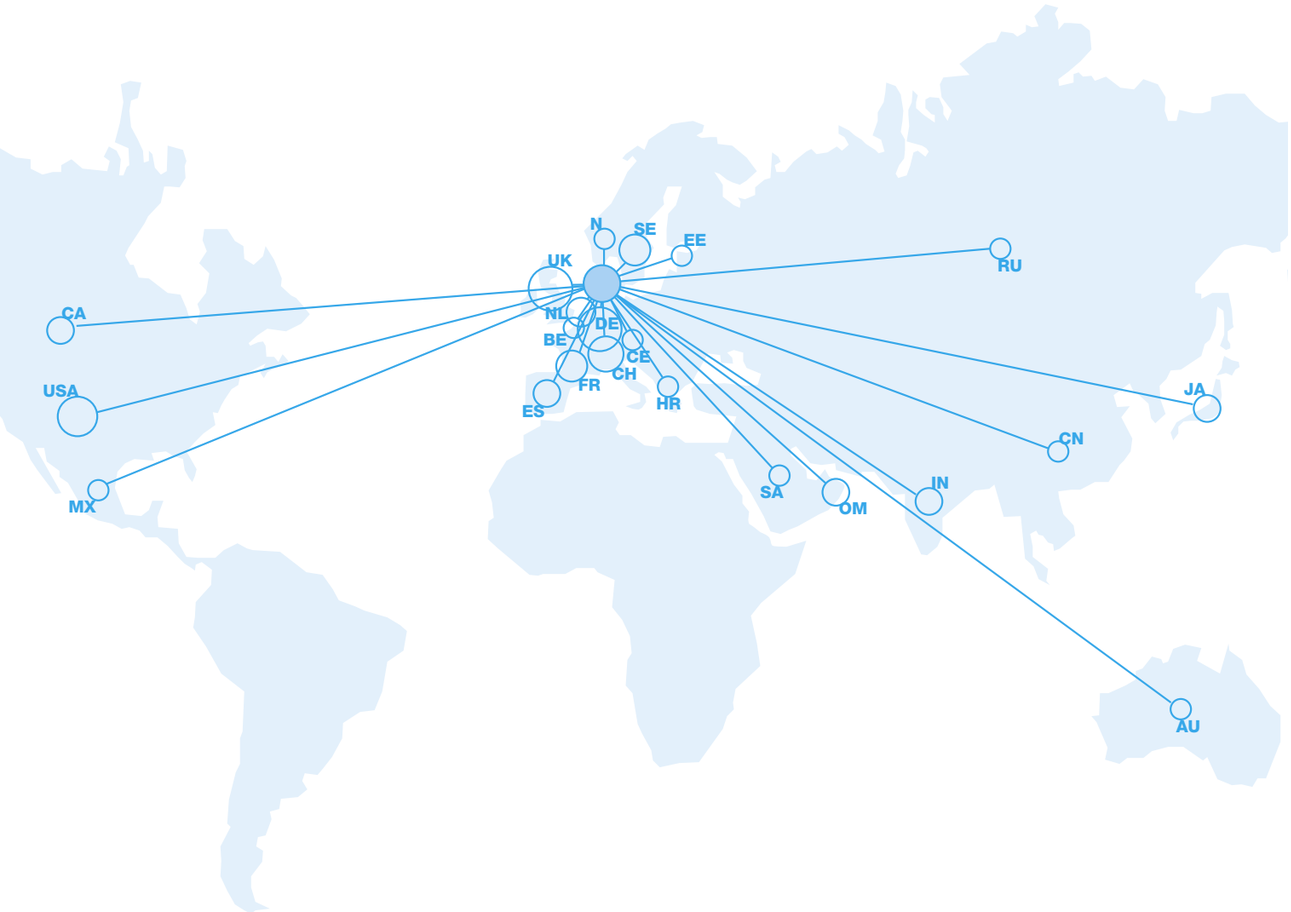
After one-and-a-half-years as a postdoc in the Ubiquitin Signaling Group at the Department of Disease Biology I have moved to Beijing Institute of Genomics, Chinese Academy of Sciences, where I have taken up a position as a visiting scientist and team leader for a small group focusing on screening for and characterizing novel proteins involved in the DNA damage response and repair.

I really enjoyed my time at CPR, where I worked with great colleagues and inspiring and knowledgeable supervisors in an ambitious, fun and energetic environment. One of the main strengths of the Center is that it encompasses different research areas and many highly competent scientists. The access to state-of-the-art technologies and leading experts within proteomics and protein production has been especially important for my projects, and luckily the collaborative nature of the Center means that I can still take advantage of these great resources.

I believe that being given the opportunity to work in different scientific environments is a privilege that makes you grow both scientifically and personally. The scientific communities and collaborations that I have gained access to will without any doubt be invaluable for my future career.

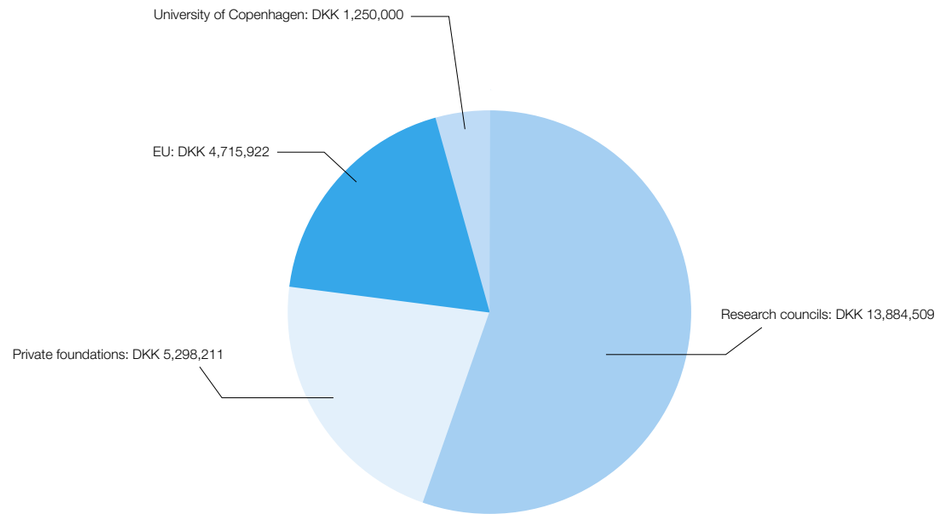
– Jannie Rendtlew Danielsen

CPR Collaborative Publications 2011



Key international collaboration partners	Joint publications
University of Oxford (UK)	6
Max Planck Institute of Biochemistry (DE)	5
University of Cambridge (UK)	4
Harvard University (US)	3
Karolinska Institute (SE)	3
University of London Imperial College (UK)	3
University of Zurich (CH)	3
Leiden University (NL)	2
Mayo Clinic (US)	2
Newcastle University (UK)	2
Sultan Qaboos University (OM)	2
Texas A&M University (US)	2

External Funding

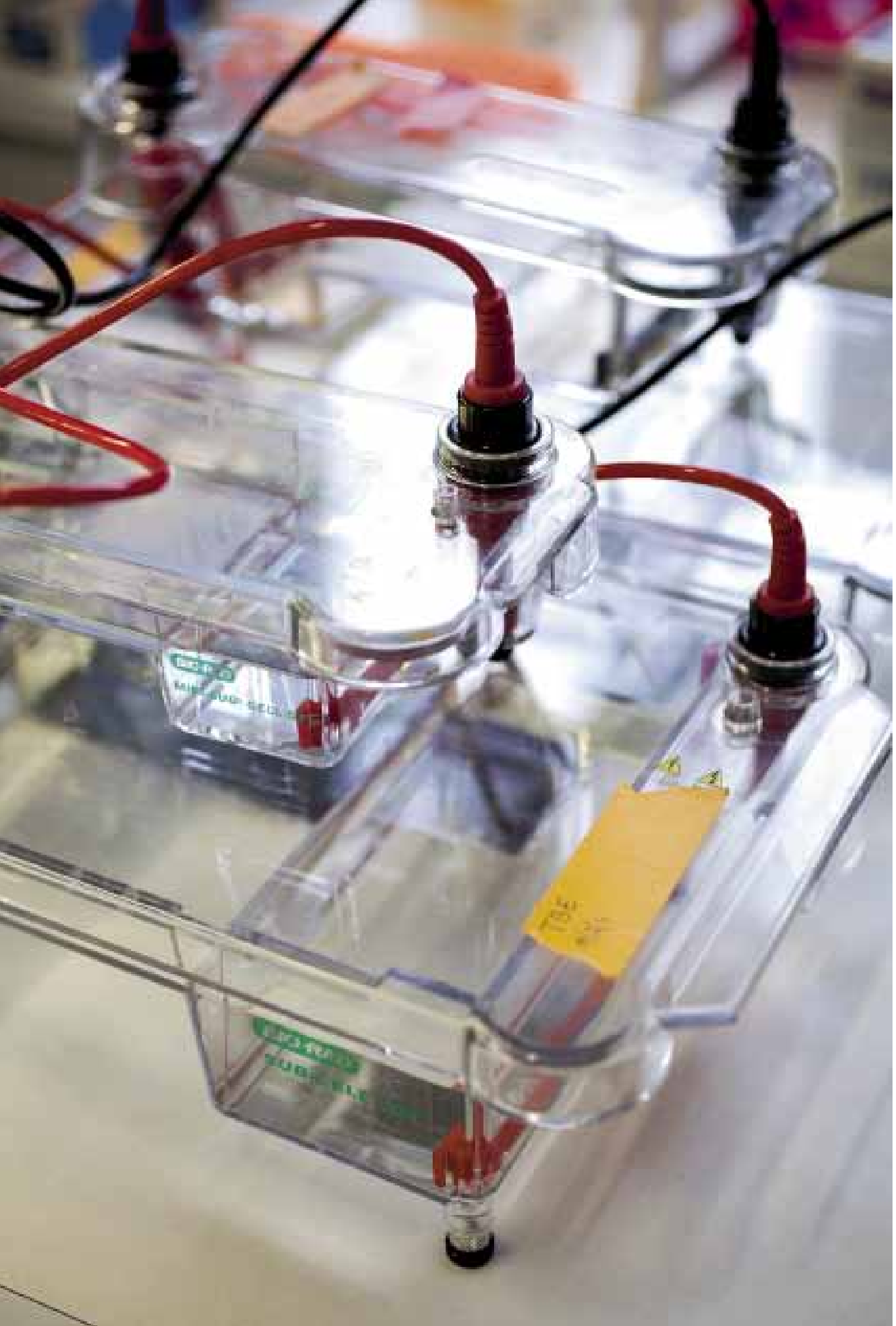


Overview of the distribution of DKK 25 million external funds granted in 2011 according to funding source.

2011 proved to be another successful year for attracting external funding to the Center. All in all, more than DKK 112 million (EUR 15 million) has been secured so far, with DKK 25 million (EUR 3.3 million) awarded this year. CPR now has two Sapere Aude: DFF Starting Grant recipients (Danish Council for Independent Research), with this year's addition, Prof. Jesper Velgaard Olsen, receiving DKK 8.1 million (EUR 1.1 million) for his research project 'Phosphoproteomics Tracing of Cancer Therapeutic Drug Effects in Signaling Networks from Tissues and Organs' (Prof. Niels Mailand received DKK 7.6 million in 2010). Additional grants from the Danish Council for Independent Research, The Lundbeck Foundation, Novo Nordisk A/S and the Chinese Academy of Science as well as NordForsk were approved for projects running for two or more years. Negotiations for one EU FP7 collaborative project (BioMedBridges) and one Marie Curie Initial Training Network project (UPStream) were finalized during 2011 and CPR expects to receive DKK 4.7 million (EUR 0.6 million) in total over the next five years for these projects. We gratefully acknowledge the match funding for the EU projects provided by the University of Copenhagen. The Center is now involved in seven EU FP7 collaborative projects in addition to one Marie Curie Fellowship.

ASSET: 'Analyzing and Striking the Sensitivities of Embryonal Tumors', is a 14 partner collaborative European FP7 - HEALTH - 2010 project involving two departments at CPR; Disease Systems Biology and Proteomics. The project started in 2011 and uses a combination of state-of-the-art genomics, proteomics and mathematical modeling, to identify mechanistically understood network vulnerabilities that can be exploited for new approaches to the diagnosis and treatment of highly aggressive and devastating major paediatric tumors. The basic hypothesis is that embryonal tumors (ETs) share common pathogenetic principles that can be captured and made accessible to rational analysis by combining high-throughput and high-content analysis of the genome, transcriptome and proteome with mathematical modeling. By focusing on unraveling the signaling networks and their alterations in ETs, the consortium hopes to *(i)* improve understanding of, and therapeutic options for, devastating childhood malignancies and *(ii)* enable a rational approach to deal with the complexity of the pathogenesis of adulthood cancers.

Two PhD students, Kristina Emdal from the Department of Proteomics and Kalliopi Popi Tsafou from the Department of Disease Systems Biology, are currently working full time on the project, giving them ample opportunities to interact with European experts within their respective areas. In addition to external funding, involvement in EU projects thus provides PhD students and postdoctoral fellows at the Center with easy and invaluable access to the elite in European research.



Community Projects

In addition to our ongoing research collaborations and those initiated by direct and more informal contacts, we also offer the opportunity to submit research proposals to our research units for subsequent formal review. We currently have ongoing projects at the Department of Proteomics and the Facility for Protein Science and Technology, and these are briefly summarized below.

Department of Proteomics

As part of the proteomics accessibility scheme, five collaborative research project proposals have been accepted during 2011. The focus of the collaborative projects falls within a broad scientific area, but they largely aim at identifying post-translational modifications (PTMs) on specific protein groups that are of scientific interest. As an example of such collaborative research proposals, there is a brief project description below. In total six proposals were submitted during 2011. The Department of Proteomics has maintained an overall high acceptance rate (~70%) for submitted research proposals since the community access scheme was initiated.

Identification of Post-translation Modifications on the Arx, Pax4 and Lim1 Proteins

Dr. Jacob Hecksher-Sørensen, Hagedorn Research Institute, Novo Nordisk, Copenhagen, Denmark

Arx and Pax4 are known to be implicated in fate determination of endocrine cells, and recently, Pax4 has been shown to convert adult alpha cells into insulin-producing beta-cells following over-expression (Collombat *et al.*, Cell, 2009). Currently no PTMs have been described on any of these proteins, and given the immense focus on reprogramming as a treatment for diabetes, it is believed that certain PTMs on the investigated proteins can be associated with a biological activity. Thus, the aim of this collaboration is to utilize the proteomics platform at CPR to identify specific PTMs implicated in the fate determination of endocrine cells, and subsequently validate the biological functions of such novel PTMs at the Hagedorn Research Institute.

Facility for Protein Science and Technology

On the basis of advice from the Community Target Committee, the Facility for Protein Science and Technology engaged in two new community collaborative projects during the year, in addition to several ongoing collaborations e.g. B-lymphoid kinase (Prof. Niels Ødum, University of Copenhagen), PDZ domains (Prof. Ulrik Gether, University of Copenhagen), chitinases (Dr. Jørgen Leisner, University of Copenhagen), cancer-related antigens (Prof. Henrik Ditzel, University of Southern Denmark) and host-pathogen interactions (Dr. Johan Malmström, Lund University, Sweden). The two community projects initiated during the year are briefly described below.

Heat Shock Factor Protein 1 and 2

Prof. Lea Sistonen, Turku Centre for Biotechnology, Åbo Akademi University, Finland

When cells are exposed to stressful situations such as elevated temperatures, heat shock transcription factors (HSFs) are activated, leading to expression of a small number of conserved proteins; the heat shock proteins (Hsps) that protect the cell from the deleterious effects of stress. This response is universal, and has been documented in every organism in which it has been sought, from prokaryotes to eukaryotes and from yeasts to mammals. In mammals, the HSF family has four members (HSF1-4), of which HSF1 and HSF2 have been implicated in the activation of Hsp-synthesis induced by stress. This collaboration will involve recombinant expression of HSF1 and HSF2 for subsequent characterization, including structural studies.

Characterization of the Receptor for Glycodelin on Human Leucocytes

Dr. Steen Sørensen, Department of Clinical Biochemistry, Hvidovre Hospital, Denmark

Several functional *in vitro* studies have demonstrated biological effects of glycodelin A, a protein mainly secreted by the endometrial glands during the menstrual cycle and through the first trimester of pregnancy. The effects comprised mostly immunological properties observed in NK-cells, B-cells, monocytes and T-cells and it has been hypothesized that glycodelin A has an important role in protecting the embryo from the maternal immune response. Despite many functional studies, the structural description of the glycodelin A receptor has not been fully elucidated and this collaboration will involve recombinant protein expression of glycodelin A to characterize its receptor on human leucocytes.

Conferences Organized by the Center

Interactions with the surrounding scientific community, local as well as international, remain high on the agenda for the Center. Thus, CPR was involved in organizing or co-organizing a considerable number of scientific conferences, symposia and workshops during the year, four of which are described in more detail below.

2nd Nordic Proteomics Symposium

The 2nd Nordic Proteomics Symposium was held at the Faculty of Health Sciences at the Panum Institute, Copenhagen on November 27-29, 2011. The Symposium was hosted by the Swedish, Danish, Norwegian, and Finnish Proteomics Societies and organized by The Swedish Academy of Pharmaceutical Sciences and Jesper Velgaard Olsen on behalf of CPR.

The overall theme of this Nordic meeting was 'Functional Proteome Research', including scientific interactions, discussions and debates, challenging the scientific frontiers in modern clinical, preclinical and biological proteomics research. Important Life Science developments and research trends like clinical proteomics, membrane proteins and transport schemes, drug target inhibition, and compound characterizations were presented, as well as pathophysiology-related and disease-related protein expression studies. The symposium drew close to 200 participants in the scientific program, and there was an extensive exhibition featuring all major vendors within Proteomics.

Jerry Workman (Stowers Institute, Kansas City, US) gave an excellent keynote lecture highlighting the history and current impact of protein analysis on chromatin biology. This was the first presentation from a list of most impressive speakers including Ruedi Aebersold (ETH Zürich, Switzerland), Leigh Anderson (NIH) and Matthias Mann (Max Planck Institute in Munich, Germany, and CPR).

A perfect mixture of lectures, abstract talks and technology developments, combined with an outstanding social program, allowed participants to get the most out of the symposium. We also gave several guided tours of the CPR facilities to the international researchers attending the symposium.

Frontiers in Cell Biology

Simon Bekker-Jensen and Niels Mailand organized the highly successful 39th Annual Meeting of the Danish Society for Biochemistry and Molecular Biology – 'Frontiers in Cell Biology' on October 26-28, 2011. Around 150 participants attended the scientific part of the program. The meeting covered a wide range of topics, including cellular responses to DNA damage, ubiquitin and ubiquitin-like modifiers, chromatin and epigenetics, cell cycle, mitosis and chromosome dynamics. An impressive line-up of internationally renowned researchers in these areas, interspersed with local experts, provided an excellent scientific program for the participants. The conference featured keynote lectures by Wade Harper (Harvard Medical School, US) and Jan-Michael Peters (IMP, Vienna, Austria), as well as excellent talks by many other top speakers, including Kim Nasmyth (University of Oxford, UK), Ivan Dikic (University of Frankfurt, Germany), and Susan Gasser (FMI, Zürich, Switzerland).

Nordic Mitosis Network

The Nordic Mitosis Network is supported by a grant from NordForsk to Assoc. Prof. Jakob Nilsson, group leader in Mitotic Mechanisms and Regulation, Department of Disease Biology, CPR. The network brings together the research groups of Jakob Nilsson, Arne Lindqvist (Karolinska Institutet, Sweden), David G. Nord (Lund University, Sweden) and Lila Kallio (VTT Technical Research Centre of Finland) with the aim of promoting research on mitosis in the Nordic countries. The network will run from 2011-2013 and it includes an annual meeting to promote interactions between the groups and share knowledge and ideas. In addition, two workshops will be arranged covering techniques and topics relevant for the participating groups.

The first annual meeting was organized by Jakob Nilsson and held at CPR on December 12, 2011, combining talks from a number of invited speakers with talks of members of the network. Invited speakers included Jon Pines (University of Cambridge, UK), Anna Santamaria (Biozentrum, Basel, Germany), Thomas Mayer (University of Konstanz, Germany) and Marcos Malumbres (Spanish National Cancer Research Centre, Spain). The meeting provided an excellent kick-start of the network and was much appreciated by the participants.

Protein Production and Purification Partnership in Europe (P4EU) Workshop

Bjørn Voldborg, Head of Protein Production Unit, the Facility for Protein Science and Technology, CPR, hosted the 1st P4EU workshop on eukaryotic protein expression platforms, from November 30 to December 1, 2011. The purpose of the workshop was to allow laboratories in the P4EU partnership to exchange knowledge and gain experience from each other. Thanks to a very informal format, the workshop ensured optimal networking and interactions combined with presentations from all participating laboratories. The goal of the P4EU is to optimize European protein production technologies by linking protein production facilities across Europe. The recent workshop set the stage for future productive collaborations within the partnership.

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Employed (110) and affiliated (22) staff as of 31 December 2011.

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Anders Hjort
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Head of Systems Administration

Gitte Thorvil
IT Engineer

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Center for Protein Research

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