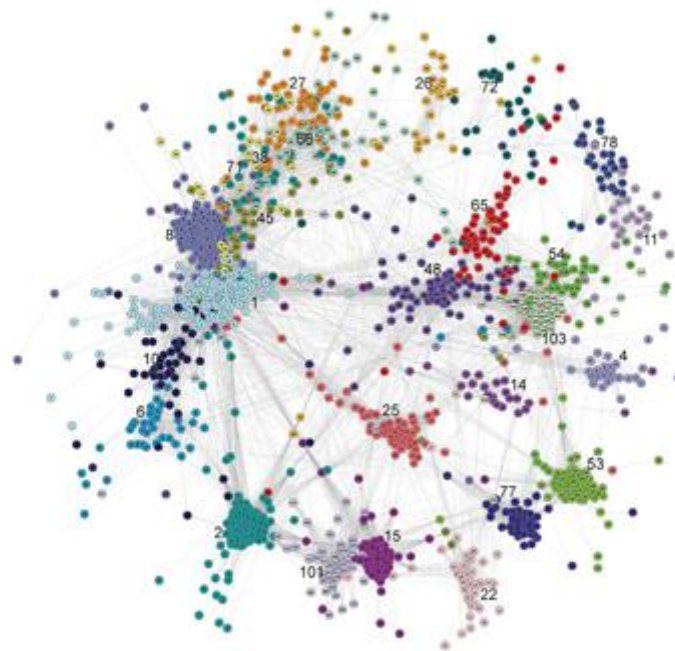


Newsletter 14

Covering the period 1 April – 30 September 2011



Identifying Correlations in Electronic Patient Records

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).

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General Update

Michael Sundström steps down from his position as Managing Director of CPR

Michael Sundström has unfortunately decided to leave his position as Managing Director at the Novo Nordisk Foundation Center for Protein Research at the University of Copenhagen, to assume the position as Vice President, Discovery Research at Karolinska Development in Stockholm from 1 October 2011. In addition to being able to work more in depth with translational research activities, it also allows him to be closer to his family in Stockholm. Michael has been working with the Center from the very early planning stage to the close to fully operational organization it is today, with high-end infrastructure and resources, as well as an excellent team of scientists and support staff.



"I have been working with this exciting project now since four and a half years, and the Center is excellently positioned as a world-leading organization within the area of protein research. Although I look much forward to my new challenges, I will certainly miss the Center and its tremendous resources, its staff, friends and collaborators" - says Michael Sundström.

During this coming period, the Dean has asked Jesper Olsen, Group Leader in the Department of Proteomics, to act in the role as the interim Managing Director for the Center, which he has accepted. Jesper was one of the first researchers hired at CPR and therefore he has considerable insights into the Center's daily operations. Jesper, who is a world leading scientist in the area of phosphoproteomics, will together with Research Directors Søren Brunak and Matthias Mann, form the new management team of the Center. Michael Sundström and Jesper Olsen have worked regularly and in detail to ensure a smooth transition period, and to set the stage for the continued success for the Center for Protein Research.

CPR would like to thank Michael Sundström for his extraordinary job in leading the Center from start-up to operations and wish him the best of luck in his position in Stockholm.

New Group Leader - Disease Biology



CPR has recently recruited one Group Leader to the Department of Disease Biology: Jakob Nilsson from the Biotech Research and Innovation Centre (BRIC), University of Copenhagen. Jakob Nilsson is head of the "Mitotic Mechanisms and Regulation" research group. In August 2011 Jakob and his team moved into their dedicated space and commenced activities at the Center laboratories.

Jakob Nilsson got his PhD in 2002 followed by a postdoctoral position with Poul Nissen at the University of Aarhus. He did his second postdoctoral fellowship in Jonathan Pine's laboratory, Gurdon Institute, University of Cambridge before joining BRIC as an independent Group Leader in March 2009.

Jakob Nilsson and his group will focus on understanding how cells achieve accurate chromosome segregation during mitosis, a process that is often defective in tumor cells. The cell employs a checkpoint referred to as the spindle assembly checkpoint to avoid errors in chromosome segregation and the aim is to obtain a molecular understanding of how this checkpoint works. Read more about Jakob Nilsson's group at: <http://www.cpr.ku.dk/groups/diseasebiology/mitoticmechanisms/>.

Awards

Søren Brunak appointed ISCB Fellow 2011

Professor Søren Brunak receives the prestigious honor of being appointed ISCB Fellow 2011. The ISCB Fellowship award is given for outstanding contributions to the fields of computational biology and bioinformatics and for having demonstrated excellence in research and served the interest of the ISCB Community.



The International Society for Computational Biology appointed four Fellows in 2011 which in addition to professor Brunak includes Michael Ashburner from EMBL-EBI, UK, Philip E. Bourner, University of California, San Diego, US, Richard Durbin, Wellcome Trust Sanger Institute, UK.

Brian T. Weinert awarded EuPA prize



Brian Tate Weinert, post-doctoral fellow in the Department of Proteomics was awarded the best oral

presentation prize at the European Proteomics Association (EuPA) day that was organized in conjunction with the Human Proteome Organization (HUPO) World Congress 2011 in Geneva, Switzerland.

Infrastructure & Equipment

During the period covered by this newsletter, key instrumentation has been ordered or installed at the Center, including a high-content screening system and high-end mass spectrometers.

DISEASE BIOLOGY: A general infrastructure upgrade of the Department of Disease Biology has been approved and planned. This will feature the acquisition of several imaging systems to upgrade our access to advanced light microscopy-based technologies not readily available at the University, as well as additional capital equipment. The microscopy suite (see selected images below) includes a Scan^R automated microscope (Olympus), which e.g. will allow the groups at CPR to perform medium- to high-throughput siRNA-based screens in an automated fashion.

Moreover, two Zeiss and a Deltavision microscope will be acquired to cover specialized needs for confocal and time-lapse microscopy, as well as enabling us to perform DNA damaging microlaser irradiation of living cells.



In addition to the acquisition of capital equipment, the existing facilities for cell culture work will be expanded considerably to accommodate the increasing need for tissue culture work throughout the department.

PROTEOMICS – NEW HIGH-END MASS SPECTROMETERS: As a continuous optimization of the integrated proteomics platform, the Department of Proteomics have upgraded its instrument park to six of the latest-generation Q-Exactive high-performance mass spectrometers (MS). These new instruments bring together quadrupole precursor selection and high-resolution accurate mass Orbitrap mass analysis, hereby providing improved speed, accuracy and sensitivity as compared to previous generations of MS. With a fast scan speed and multiplexing capabilities, the Q Exactive mass spectrometer is an ideal MS instrument for in-depth peptide sequencing. To match the fast sequencing speed of the Q-Exactive the department has also purchased two Ultra-high performance liquid chromatography (UHLPC) systems for improved chromatography performance. With the purchase of these instruments the Department of Proteomics extends its proteomics sequencing capacity, and furthermore ensures that the department keeps its competitive scientific edge.



Updates from Departments and Groups

Protein Production Unit (PPU)

As a result of the work focusing on development of high-throughput (HT) eukaryotic expression systems, we have now further expanded the HEK293-EBNA based HT platform by constructing 19 new vectors. Thus, we can now provide HEK expressed proteins with distinct combinations of identification and purification tags. Current efforts focus on establishing HEK293 secretory and co-expression platforms.

The publicly available CPR Protein Resource (<http://cpr1.sund.ku.dk/cgi-bin/cpr.pl>) now contains samples from 208 protein batches representing 144 unique protein targets, all made available to the scientific community.

Collaborations: The PPU is a partner in the EU F7P Affinomics project focused on developing better affinity binders, and has over the past six months, provided more than 100 protein and DNA samples to our partners to be used as antigens for the generation of improved binders. In addition, as part of our collaboration with Professor Ian Wilson at SCRIPPS (San Diego, US), 27 protein samples were produced for structural X-ray studies, resulting in two solved structures so far. A collaboration with the Novo Nordisk Foundation Center for Basic Metabolic Research (University of Copenhagen) has been initiated focused on purification and functional characterization of GPCR receptors and their domains. In collaboration with Professor Ulrik Gether (University of Copenhagen) we have produced a large number of PDZ domain containing proteins. Our project on expression and structural analysis of membrane proteins has progressed into a collaboration with Professor Poul Nissen's group at the Center for Structural Biology, University of Aarhus, where protein crystallization efforts are ongoing.

Staff: Since 1 September, Tine Kragh Nielsen, previously employed as a postdoc in the PPU, has taken on the responsibility as Team Leader for the Protein Purification Team. A new laboratory technician trainee has started in PPU following Farooq Gulomar who successfully passed his exam.

Chemical Biology Unit

The Chemical Biology Unit has worked on finalizing the automatic liquid and robotic handling of our screens using an integrated platform from Caliper in addition to the automated storage of the CPR compound collection.

Collaborations: We have initiated collaboration with the NIH Chemical Genomics Center (NCGC), Bethesda, US, focusing on ADAM proteases. Furthermore, in collaboration with Dr Stefan Knapp, University of Oxford, we have profiled a number of PIM mutants against a panel of PIM inhibitors.

Our chemical compound collection has grown with new sub libraries specifically targeting kinases and proteases. Through our collaboration with Prof. Wang at the National Center for Drug Screening in Shanghai, ~50 novel protease inhibitors have been synthesized.

Staff: Olivier Bitterlin, Laboratory/Service Engineer, started 1 July. Rosa Rakownikow Jersie-Christensen started 1 July, as a temporary scientific assistant working within the Bioassay group. Simon Brown, M.Sc. student, started 1 September working together with Alexander Kotsch in setting up expression methods and purification of various domains of ADAM proteases. Anne Sofie Molsted Wanscher completed her M.Sc. thesis entitled “Solubility and half-life improvements of proteins with therapeutic potential”.

Molecular Endocrinology Group

This summer, Søs Mathiasen successfully completed her research project required for her BSc degree. Stine Kundby Smedegaard completed her MSc thesis project entitled: “Functional characterization of Asb5, As13 and Cullin 5 and their significance in prostate cancer cell proliferation and invasion”. Stine will join the group as research assistant starting 1 November.

Scientific Advisory Board – Members

It is with regret we announce that Prof. Peer Bork, Joint Head of the Structural and Computational Biology Unit, EMBL, has stepped down from his position as a member of the Scientific Advisory Board in May 2011. We would like to thank Prof. Bork for his time and valuable contribution to our Scientific Advisory Board. The search for a replacement is ongoing.

Staff & Recruitment

Recruitment activities continue to be a high priority at CPR. Overall, good progress is made regarding additions of staff members to all established groups. Of particular focus to the Center management team was the addition of two additional Group Leaders in Disease Biology. Besides Associate Prof. Jakob Nilsson who already started up with his group at the Center we have also recruited Dr. Jeremy Daniel who is currently at NIH in Bethesda, US. Jeremy will join CPR from 1 January 2012. By the end of September, CPR had 130 employees of which 46 were non-Danish representing 25 different nationalities.

New members joining the CPR during the period 1 April to 30 September 2011 were:

Disease Systems Biology:

Kalliopi Tsafou – Research Assistant
Jan Refsgaard Nielsen – Research Assistant

Mass spectrometry for quantitative proteomics Group:

Louise Knudsen – STAR Postdoc (employed at Novo Nordisk)
Kristina Emdal – PhD Student

Proteomics and Cell Signaling Group:

Amit Kumar – Research Assistant

Mitotic Mechanisms and Regulation Group:

Jakob Nilsson – Group Leader and Associate Professor
Daniel Hayward – Postdoc
Gang Zhang – Postdoc
Garry Sedgwick – Postdoc
Tiziana Lischetti – PhD Student
Julie Schou – PhD Student
Jamin Hein – Master Student

Ubiquitin Signaling Group:

Ian Gibbs-Seymour – Postdoc
Yasuyoshi Oka – Postdoc
Berthe Fiil – Postdoc
Bine Villumsen – Research Assistant
Rebecca Kring Hansen – Master Student
Christina Råe Hansen – Student Assistant

Chemical Biology Unit:

Olivier Bitterlin – Service Engineer
Rosa Jersie-Christensen – Research Assistant
Simon Brown – Master Student

Protein Production Unit:

Simon Danyel Graves – Laboratory Technician Trainee

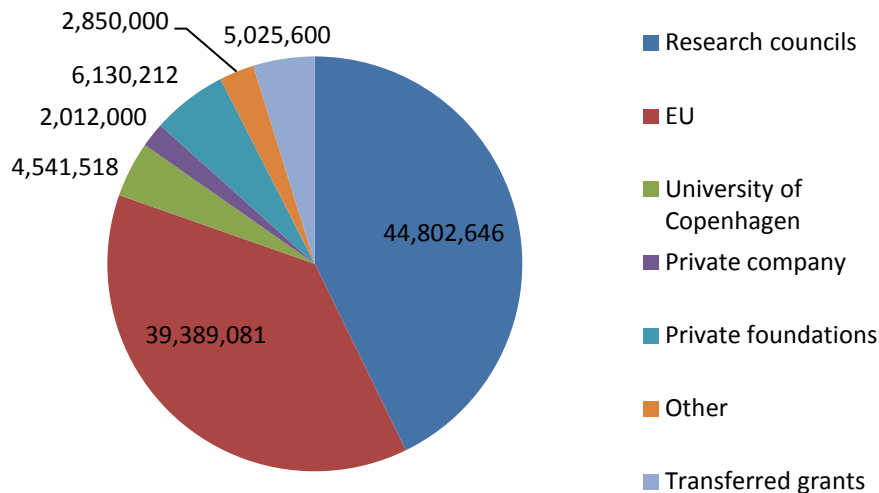
External Funding

Scientists at CPR continue to attract external funding in addition to the donation from the The Novo Nordisk Foundation. We have to date (30 September 2011) secured a total of ~104 MDKK in external funding, with the following grants recently approved:

- Jesper Velgaard Olsen received a Sapere Aude: DFF Starting Grant from the Danish Council for Independent Research - Medical Sciences. He receives more than 8.1 MDKK for his project entitled: *'Phosphoproteomics Tracing of Cancer Therapeutic Drug Effects in Signaling Networks from Tissues and Organs'*.
- In addition, Jesper was awarded 250 kDKK from the Faculty of Health Sciences (Proteomics).
- Amilcar Flores Morales received a grant (2.6 MDKK) from the Danish Council for Independent Research - Medical Sciences for the project *'The significance of SOCS2 for the aetiology of non-alcoholic steatohepatitis'* (Molecular Endocrinology).
- Dr. Gang Zhang from Jakob Nilsson's group received a 2 year postdoctoral fellowship (1,8 MDKK) from the Danish Council for Independent Research - Medical Sciences (Mitotic Mechanisms and Regulation).



- Jakob Nilsson received a NordForsk grant to establish the “Nordic Mitosis Network” (0.7 MNOK). The first annual meeting of the network will be held at CPR on 12 December featuring a number of invited speakers.
- Negotiations for one EU-FP7 collaborative project are under way and CPR expects to receive ~350 k€ for the project BioMedBridges (Disease Systems Biology) to commence in 2012. In addition another ~320 k€ is expected for the participation in the EU-FP7 Marie Curie Initial Training Network project UPStream (Proteomics).



Overview of approved external funding including transferred grants by 30 September 2011 (DKK).

Seminar Series

The CPR seminar series continued, with the following appreciated presentations:

- **15 April 2011:** Prof. Christopher Schofield
Department of Chemistry, University of Oxford, Oxford, U.K. *Molecular basis of oxygen sensing in humans and other animals*
- **18 May 2011:** Prof. Morten Meldal
Nano Science Center, University of Copenhagen. *Enzyme Reactions in Beaded PEG Gel-Resins*
- **25 May 2011:** Prof. Pernille Rørth
Institute of Molecular and Cell Biology (IMCB), A-STAR, Singapore. *Guidance of collective cell migration*
- **15 June 2011:** Prof. Torben Ørntoft
Head of Department of Molecular Medicine, Aarhus University Hospital at Skejby, Denmark. *Translational research in oncology: Molecular determination of prognosis, and prediction of response*

- **14 September 2011:** Prof. Lars Björck
Division of Infection Medicine (BMC), Lund University, Sweden. *Streptococcus pyogenes, proteolysis and innate immunity*

The following are confirmed speakers to conclude the autumn 2011 CPR seminar series talks:

- **12 October 2011:** Assoc. Prof. Jakob Nilsson
Mitotic Mechanisms and Regulation, Department of Disease Biology, CPR
- **22 November 2011:** Dr. David Komander
MRC Laboratory of Molecular Biology, Cambridge, U.K.
- **7 December 2011:** Dr. Francesca Ciccarelli
Evolutionary Genomics of Cancer, IFOM-IEO-Campus, Milan, Italy

For additional information see <http://www.cpr.ku.dk/seminars/>

The CPR seminar series is from now on organized by the PhD students at the Center which promises an exciting speakers list for 2012.

Publications

So far we have published more than 80 papers with CPR author affiliation.

From April - September 2011:

- **Cdc20 control of cell fate during prolonged mitotic arrest.**
Jakob Nilsson
Bioessays, in press
- **Structure of a Blinkin-BUBR1 complex reveals an interaction essential for kinetochore-mitotic checkpoint regulation underlying an unanticipated binding site.**
Victor M. Bolanos-Garcia*, Tiziana Lischetti, Dijana Matak-Vinković, Ernesto Cota, Pete J. Simpson, Dimitri Y. Chirgadze, David R. Spring, Carol V. Robinson, Jakob Nilsson, and Tom L. Blundell*
Structure, in press
- **Ten Simple Rules for Getting Help from Online Scientific Communities.**
G.M. Dall’Olio, J. Marino, M. Schubert, K.L. Keys, M.I. Stefan, C.S. Gillespie, P. Poulain, K. Shameer, R. Sugar, B.M. Invergo, L.J. Jensen, J. Bertranpetit and H. Laayouni
PLoS Computational Biology, e1002202, 2011
- **Using Electronic Patient Records to Discover Disease Correlations and Stratify Patient Cohorts.**
F.S. Roque, P.B. Jensen, H. Schmock, M. Dalgaard, M. Andreatta, T. Hansen, K. Søbey, S. Bredkjær, A. Juul, T. Werge, L.J. Jensen, and Søren Brunak
PLoS Computational Biology, 8, e1002141, 2011

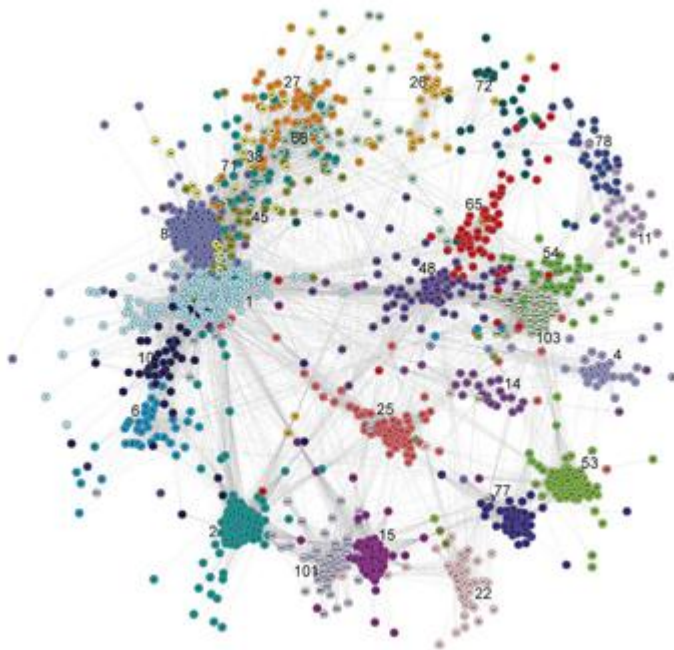
- **The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks.**
Bekker-Jensen S, and Mailand N. (2011).
FEBS Letters 18, 2914-2919.
- **The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks.**
Meerang M, Ritz D, Paliwal S, Garajova Z, Bosshard M, Mailand N, Janscak P, Hübscher U, Meyer H, and Ramadan K. (2011).
Nature Cell Biology, in press.
- **A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles.**
Wagner SA, Beli P, Weinert BT, Nielsen ML, Cox J, Mann M, Choudhary C.
Mol Cell Proteomics. 2011 Sep 1. [Epub ahead of print]
- **A phospho-proteomic screen identifies substrates of the checkpoint kinase Chk1.**
Blasius M, Forment JV, Thakkar N, Wagner SA, Choudhary C, Jackson SP.
Genome Biol. 2011 Aug 18;12(8):R78. [Epub ahead of print]
- **Proteome-wide mapping of the Drosophila acetylome demonstrates a high degree of conservation of lysine acetylation.**
Weinert BT, Wagner SA, Horn H, Henriksen P, Liu WR, Olsen JV, Jensen LJ, Choudhary C.
Sci Signal. 2011 Jul 26;4(183):ra48.
- **Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth.**
Wild P, Farhan H, McEwan DG, Wagner S, Rogov VV, Brady NR, Richter B, Korac J, Waidmann O, Choudhary C, Dötsch V, Bumann D, Dikic I.
Science. 2011 Jul 8;333(6039):228-33. Epub 2011 May 26.
- **The SOCS2 ubiquitin ligase complex regulates GH receptor level.**
Vesterlund M, Fahad Al-Zadjali, Persson T, Kessler BM, Nielsen ML, Norstedt G and Flores-Morales A
PLOS One, 2011
- **A Liver X Receptor agonist down-regulates Growth Hormone signaling in the liver.**
Fahad Zadjali, Ruyman Santana-Farre, Ewa Ellis, Gunnar Norstedt, Leandro Fernandez-Perez and Amilcar Flores-Morales.
Hormone Molecular Biology and Clinical Investigation. Oct 2011. In press.
- **RNA-DNA sequence differences (RDD) spell genetic code ambiguities?**
Bentin T and Nielsen ML
Artificial DNA, 2011
- **Pinpointing phosphorylation sites: Quantitative filtering and a novel site-specific x-ion fragment.**
Kelstrup CD, Hekmat O, Francavilla C, Olsen JV.
J Proteome Res. 2011 Jul 1;10(7):2937-48. Epub 2011 Apr 28.

- **Phosphorylation of the yeast γ -tubulin Tub4 regulates microtubule function.**
Lin TC, Gombos L, Neuner A, Sebastian D, Olsen JV, Hrlle A, Benda C, Schiebel E.
PLoS One. 2011 May 5;6(5):e19700.
- **GeLCMS for in-depth protein characterization and advanced analysis of proteomes.**
Lundby A, Olsen JV.
Methods Mol Biol. 2011;753:143-55.
- **Separation of the gluconeogenic and mitochondrial functions of PGC-1 α through S6 kinase.**
Lustig Y, Ruas JL, Estall JL, Lo JC, Devarakonda S, Laznik D, Choi JH, Ono H, Olsen JV, Spiegelman BM.
Genes Dev. 2011 Jun 15;25(12):1232-44. doi: 10.1101/gad.2054711. Epub 2011 Jun 6.

Research Highlights

DISEASE SYSTEMS BIOLOGY: Identifying Correlations in Electronic Patient Records

A new study demonstrates 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, has been published in the open-access journal *PLoS Computational Biology* on 25 August 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 also may be very valuable for choosing personalized treatment regimes", says Professor Søren

Brunak from the Technical University of Denmark and the University of Copenhagen who led the team behind the research project.

The team used the "International Classification of Disease" terminology, maintained by the WHO as a controlled vocabulary, as the basis for the analysis. "The fact that terminologies like ICD have been translated word by word between languages makes it possible in principle to use the same term profiles across language barriers and combine cohorts across countries" says author Professor Lars Juhl Jensen from the University of Copenhagen.

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 to the genetic level by investigating gene overlaps in protein interaction networks already linked to the individual diseases. "The aim here is to discover a possible genetic cause behind the disease correlations observed, thus interfacing the electronic patient record data directly to the DNA sequencing of human individuals", says Brunak.

Citation: Roque FS, Jensen PB, Schmock H, Dalgaard M, Andreatta M, et al. (2011) Using Electronic Patient Records to Discover Disease Correlations and Stratify Patient Cohorts. PLoS Comput Biol 7(8): e1002141. doi:10.1371/journal.pcbi.1002141

PROTEOMICS: 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 clearly emerging that ubiquitin also plays many other important functions in cells. However, precise mapping of modification sites and their quantification has remained a major challenge.

The Department of Proteomics (Sebastian A. Wagner, Petra Beli, Brian T. Weinert, Michael L. Nielsen, Matthias Mann and Chunaram Choudhary) recently published the largest ubiquitylation dataset in 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 map more than 11,000 endogenous putative ubiquitylation sites, of which over 90% represent novel previously non-reported sites. Previously researchers from the Center had used another affinity-purification based approach to catalogue then the 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. They show that ubiquitylation targets proteins involved in all major cellular functions and report that nearly half of all sites were shown to have non-proteasomal

functions. They conclude 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.

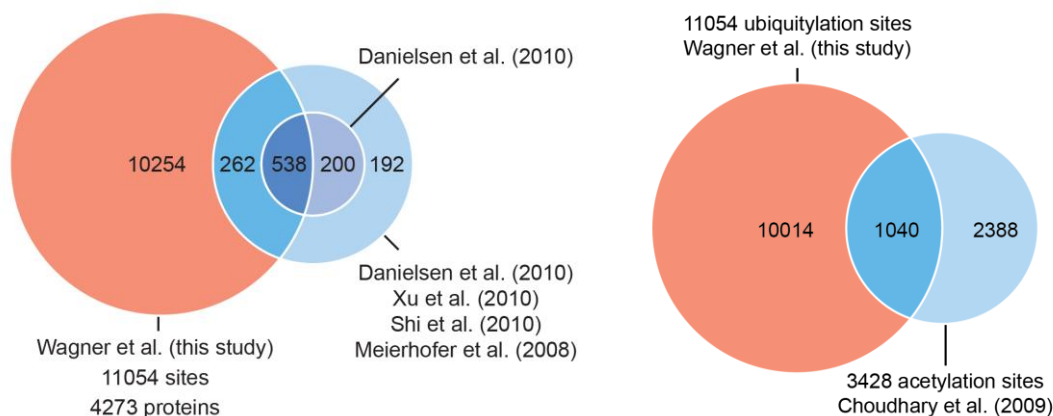


Figure: An overview of ubiquitylome and its overlap with acetylome. The left panel shows number of ubiquitylation sites identified in the current study and their overlap with previously known sites. The right panel shows substantial overlap between ubiquitylation sites with known acetylation sites in human cells.

Citation: Wagner SA, Beli P, Weinert BT, Nielsen ML, Cox J, Mann M, Choudhary C. A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. *Mol Cell Proteomics*. 2011 Sep 1.

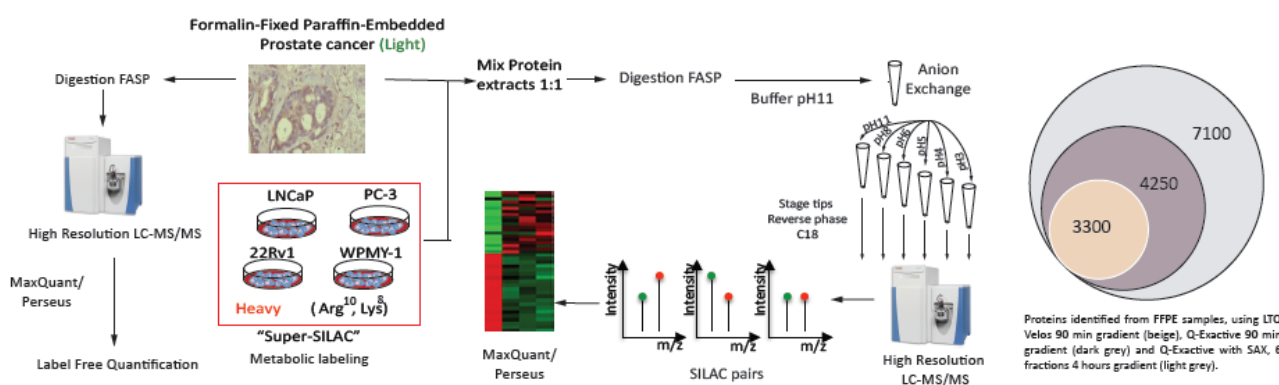
DISEASE BIOLOGY: Quantitative proteomic profiling of Formalin-Fixed Paraffin-Embedded Prostate Cancer tissue samples.

The study of proteome changes in prostate cancer (PCa) in relation to clinically significant variables, such as disease-related mortality or metastatic progression can help to define molecular predictors of disease outcome as well as increase our understanding of the mechanisms governing disease development. Because of the long natural history of this disease, where 10-15 years elapse from first indication of prostate cancer to the patient death; many important samples exist only as Formalin-Fixed Paraffin-Embedded (FFPE) specimens, which until recently, were non-compatible with in depth proteomic analysis. We have started a collaborative project with Assoc. Prof. Pernilla Wikstrom from UMEA University and Prof. Matthias Mann at the Max Planck Institute for Biochemistry in Munich to develop a methodology for quantitative proteome profiling of FFPE samples and evaluated its potential for prostate cancer research. In this study we are using recently described mass spectrometry based methodologies developed by Prof. Mann. We use the filter-assisted sample preparation method¹ to prepare protein extracts obtained from FFPE samples prior to mass spectrometry analysis. A SILAC labeled peptide mix was generated from four different prostate cancer cell cultures and spiked into samples to allow accurate peptide quantitation upon

mass spectrometry analysis². The LTQ-Orbitrap Velos and the Q-Exactive mass spectrometer were used to profile the expression of thousands of proteins in clinical FFPE prostate cancer specimens from localized and metastatic tumors as proof of principle (Figure 1).

Comparison of profiles from cryo-preserved and FFPE material from the same tumor show excellent concordance, with more than 95% of the proteins identified in both tissue samples showing correlation coefficients of 0.9 when expression levels are compared. Unsupervised clustering analysis demonstrates that protein expression profiles are sufficient to identify whether the sample originates from localized or metastatic tumors in the absence of any prior information. Functional annotation show that a wide variety of signaling pathways and functional classes are represented in these protein profiles, with no obvious bias towards specific cellular compartments. When this analysis was performed on the subset of proteins differentially expressed in metastatic vs. localized tumors we observed up-regulation of proteins involved in cell cycle, mitosis and DNA metabolism, categories also identified by gene expression profiling. On the other hand some significant discordance between gene expression data and protein levels were also evident. In summary, LC-MS/MS based quantitative proteomic profiling of FFPE samples is a powerful method for the identification of biomarkers and of novel mechanisms driving PCa cancer progression.

Figure 1. Methodology for the proteomic analysis of Formalin-Fixed Paraffin-Embedded Prostate cancer samples



References:

1. Ostasiewicz, P., D. F. Zielinska, M. Mann and J. R. Wisniewski, 2010 Proteome, phosphoproteome, and N-glycoproteome are quantitatively preserved in formalin-fixed paraffin-embedded tissue and analyzable by high-resolution mass spectrometry. *J Proteome Res* **9**: 3688-3700.
2. Geiger, T., J. Cox, P. Ostasiewicz, J. R. Wisniewski and M. Mann, 2010 Super-SILAC mix for quantitative proteomics of human tumor tissue. *Nature Methods* **7**: 383-385.