DTU

11th Workshop in Protein.DTU for Young Scientists

12 May 2014

REG-no. DK 30 06 09 46

DTU

1. Program

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11th Workshop in Protein.DTU for Young Scientists

12 May 2014
Building 306, Lecture Hall 31

08:30 - 09:00 **Arrival & Breakfast**

09:00 - 09:10 **Birte Svensson** (Professor, DTU Systems Biology)
Welcome

Chair: Birte Svensson (Professor, DTU Systems Biology)

09:10 - 09:40 **Kresten Lindorff-Larsen** (Associate Professor, Department of Biology, Copenhagen Biocenter, University of Copenhagen)
Exploiting experiments in simulations of proteins — *and perhaps giving something back*

Chair: Christine Finnie

09:40 – 10:10 **Martin Røssel Larsen** (Professor, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense)
Comprehensive quantitative proteomics and PTMomics applied to studying signal transduction pathways

Chair: Maher Abou Hachem (Associate Professor, DTU Systems Biology)

10:10 – 10:40 **Mats Ohlin** (Professor, Department of Immunotechnology, Lund University)
Evolution of carbohydrate binding proteins for enhanced performance

10:40 – 11:05 **Coffee Break**

Chair: Jenny Emnéus (Professor, DTU Nanotech), Michael Engelbrecht (Associate Professor, DTU Food)

11:05 – 12:00 **FLASH**
3 min each

12:00 – 13:00 **Lunch and poster viewing**

Chairs: Flemming Jessen (Senior Scientist, DTU Food), Peter Heegaard (Professor, DTU Vet)

12:45 – 13:30 **FLASH**
3 min each

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Chair: Pernille Harris (Associate Professor, DTU Chemistry)

13:30 – 14:00 **Jens E. Nielsen** (Senior Manager, Novozymes A/S, Bagsværd)

Deciphering fundamental properties of proteins using protein engineering: electrostatics, stability and enzymatic catalysis

Chair: Günther Peters (Associate Professor, DTU Chemistry)

14:00 – 14:30 **Mads Hartvig Clausen** (Professor, Center for Nanomedicine and Theranostics, DTU Chemistry, Kgs. Lyngby)

Probing Protein Function with Small Molecules

Chair: Peter Heegaard (Professor, DTU Vet)

14:30 – 15:00 **Henrik Ipsen** (Senior Scientist, Biochemistry, ALK-Abelló A/S, Hørsholm)

Proteins in allergenic extracts: from research to pharmaceuticals

15:00 – 16:30 Chairs: Kristoffer Almdal, Flemming Jessen, Maher Abou Hachem

Poster session, coffee and "time for a beer"

DTU

Abstract

No.

1. Avishek Majumder: Differential abundance of proteins in *Lactobacillus acidophilus* NCFM grown on raffinose
2. Bala Krishna: Critical role of a conserved transmembrane lysine in substrate recognition by the proton-coupled oligopeptide transporter YjdL
3. Casper Wilkens: Surface binding sites within α-glucan active enzymes
4. Chiara Canali: Multiplexed electrical impedance-based sensing technologies for bioengineering applications
5. Dimitrios Spanos: Motility of muscle-fibres expressing different myosin isoforms under oxidative conditions, using an in-vitro motility assay
6. Eva Krammer Andresen: Evolution of *Pseudomonas aeruginosa* regulatory proteins shapes new routes of gene regulatory networks
7. Giuseppe Dionisio: LC-MS based differential proteomics and metabolomics in cereal research
8. Hasan Ufuk Celebioglu: Proteomic Studies of Surface, Secreted, and Membrane Proteins of *Lactobacillus acidophilus* NCFM and Effects of Potential Prebiotics on Adhesion
9. Jakob Skjold-Jørgensen: Altering the activation mechanism of *Thermomyces lanuginosus* lipase
10. Johnny Birch: HEXPIN: Hetero-exopolysaccharide – milk protein interactions
11. Lila Yang: Structural Studies of Protein-Protein Interactions: FLRT Interactions with the FGF Receptor
12. Marie Sofie Møller: Design of proteinaceous inhibitors of industrially important starch degrading enzymes and proteases
13. Martin Carlsen: Designing knowledge based potentials with near-native protein funnels by semi-definite optimization
14. Martin Rykær: Protein carbonylation in bacteria and disease
15. Mikael Engmark: Development of a recombinant antibody-based treatment of snakebites
16. Morten Ejby: Oligosaccharide uptake in *Bifidobacterium animalis* subsp. *Lactis*
17. Naleli Vad: Purification and inactivation of Clostridium difficile Toxins A and B
18. Nicklas Skjoldager: Characterization of the thioredoxin system in *Lactococcus lactis*
19. Saswati Chakladar: Activity-Based Protein Probes for Investigation of Lysine Acylation
20. Sonia Al-qadi: An insect-based model for drug permeability screening across the blood brain barrier: Transcriptomic and functional analysis
21. Stinne Kirketerp Nielsen: Structural studies of β(1,6)-galactosidase from *Bifidobacterium animalis* subsp. *lactis* Bl-04 from the GH42 family in complex with small ligands
22. Susan Andersen: Biochemical characterisation of a GH62 α-L-arabinofuranosidase from *Aspergillus nidulans*
23. Susanne Jacobsen: Amino acid analysis, protein sequencing and mass spectrometry
24. Thanos Arvanitidis: Novel biomarkers for change in muscle mass or muscle pathology
25. Zacharias Brimmes Visby Damholt: Decoding Nitrosylation in Bacteria and Humans

Abstract No. 1

Differential abundance of proteins in *Lactobacillus acidophilus* NCFM grown on raffinose

Avishek Majumder[1], Liyang Cai[1], Kristian Thorsen[1], Morten Ejby[1], Sampo Lahtinen[2], Susanne Jacobsen[1] and Birte Svensson[1]

[1]*Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Søltofts Plads, Building 224, DK-2800 Kgs. Lyngby, Denmark.*
[2]*DuPont, Health & Nutrition, Sokeritehtaantie 20, 02460 Kantvik, Finland.*

Brief description of research area:

Non-digestible oligosaccharides are energy sources for the microorganisms in GIT. They can have important health benefits for human by stimulating growth of beneficial bacteria. Raffinose is one of the potential prebiotics; trisaccharide composed of galactose, fructose, and glucose. It has been shown to stimulate the growth of beneficial probiotic *Lactobacillus acidophilus* (NCFM) [1]. This study aims to identify changes in proteome of NCFM and its adhesion to mucins by the utilization of potential prebiotic oligosaccharide raffinose. For proteome analysis, the whole cell extracts were separated using two-dimensional differential electrophoresis. Protein identifications of differentially abundant spots were performed by using mass spectrometry (MS) [1]. A total of 108 protein spots, 63 spots were identified by mass spectrometry. Forty eight protein spots showed higher, while 15 showed lower relative abundance. The 63 identifications corresponding to 43 unique proteins are distributed among 12 functional categories. Several glycoside hydrolases fold changes were identified to be differentially abundant with growth of NCFM on raffinose compared to glucose. These include α-galactosidase, β-galactosidase large and small subunit, and sucrose-6-P hydrolase. Raffinose in NCFM induces the raffinose operon, consisting of genes encoding ABC transporter, α-galactosidase and sucrose phosphorylase [5].

What we know:

Differentially expressed proteins (7%) are in the category of cell envelope. This indicates that raffinose can alter the adhesion properties of NCFM.

What we need:

1. Methods to study cell surface morphology changes with advanced microscopy techniques.
2. Effect of raffinose on cell viability compared to glucose using fluorescence spectroscopy
3. Mucin interactions with cell surface of NCFM

References:

1. Majumder, A. et al. (2011).Proteome reference map of Lactobacillus acidophilus NCFM and quantitative proteomics towards understanding the prebiotic action of lactitol. *Proteomics, 11,* 3470-3481.

Abstract No. 2

Abstract template

Title: Critical role of a conserved transmembrane lysine in substrate recognition by the proton-coupled oligopeptide transporter YjdL

Johanne M. Jensen, Nanda G. Aduri, Bala K. Prabhala, Rasmus Jahnsen, Henrik Franzyk, and Osman Mirza

Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Brief description of research area:

The *Escherichia coli* genome contains four genes, *ydgR, yhiP, yjdL* and *ybgH,* that encode proton coupled di- and tripeptide transporters (POTs). A prototypical POT utilizes the secondary energy contained within the proton electrochemical gradient to accumulate di- or tripeptides inside the cell. The POTs belong to a subfamily of the functionally vast Major Facilitator Superfamily (MFS).

What we know:

Changing the highly conserved active site Lys117 in the *E. coli* POT YjdL to glutamine resulted in loss of ligand affinity as well as inability to distinguish between a dipeptide ligand and the corresponding dipeptide amide. The radically changed pH_{Bulk} profiles of Lys117Gln and Lys117Arg indicate a role for Lys117 in facilitating protonation of the transporter; a notion that is supported by the close proximity of Lys117 to the conserved EXXER POT motif previously shown to be involved in proton translocation.

What we need:

These results point towards a novel dual role of Lys117 in direct or indirect interaction with both proton and peptide.

Abstract No. 3

Title: Surface binding sites within α-glucan active enzymes

CASPER WILKENS[1,5], DARRELL COCKBURN[1], DAVID MEEKINS[2] JOSE A. CUESTA-SEIJO[3], CHRISTIAN RUZANSKI[3,4], MONICA M. PALCIC[3], ALISON SMITH[4], MAHER ABOU HACHEM[1], MATTHEW GENTRY[2] & BIRTE SVENSSON[1]

[1] *Enzyme and Protein Chemistry, Systems Biology, Technical University of Denmark, Lyngby, Denmark*

[2] *Department of Molecular and Cellular Biochemistry, College of Medicine, University of Kentucky, Lexington, Kentucky, United States of America*

[3] *Carlsberg Laboratories, Copenhagen, Denmark*

[4] *Department of Metabolic Biology, John Innes Center, Norwich, United Kingdom*

[5] *Correponding author: cwil@bio.dtu.dk (CW)*

Surface binding sites (SBS) accommodating carbohydrate ligands outside of the active site region of the catalytic domain have been observed in several crystal structures; however, only in a few cases have these SBS been characterized and shown to have an impact on the enzyme's functionality (COCKBURN & SVENSSON, 2013). The understanding of these SBSs is currently at a level where their presence is difficult, if not impossible, to predict in the absence of structural evidence. The limited data on SBSs precludes a generalisation and may mask the significance of these sites in catalysis.
We are using *e.g.* affinity gel electrophoresis (AE) and surface plasmon resonance (SPR) to both screen carbohydrate active enzymes for SBSs and characterize SBSs have been found.
Starch Excess4 (SEX4) and Like Sex Four2 (LSF2) from *Arabidopsis thalliana* are glucan phosphatases that dephosphorylate starch. LSF2 lack the CBM48 that SEX4 has, however, a crystal structure of LSF2 showed that two SBSs are present (VANDER KOI *et al.* 2010; MEEKINS *et al.* 2013). Binding studies using SPR showed that LSF2 and SEX4 had similar affinities for β-cyclodextrin (BCD). If both SBSs and the active site of LSF2 were knocked out binding to BCD was lost, while only minor decreases in the affinity were observed when the sites were knocked out individually. AE on amylopectin have shown that knocking out SBS2 resulted in complete loss of binding to amylopectin, while knocking out SBS1 and the active site resulted in a decrease in affinity.
At least one enzyme of GH77 is known to contain an SBS (FUJI *et al.* 2007) and there is likely to be others. MalQ is a α-1,4-glucanotransferase from *Escherichia coli*. Binding studies using SPR indicated that MalQ and disproportionating enzyme 2 (DPE2) (a homolog from *A. thaliana*) had similar affinities for BCD despite the fact that MalQ lacks CBMs and DPE2 has two CBM20s. Furthermore, SPR with malto-oligosaccharides showed that those of >DP4 bound with stoichiometry >1, binding at the second site being subject to competition with β-cyclodextrin. Investigations are underway to ascertain the location and function of this potential SBS in MalQ.
Starch syntase I (SSI) from Barley belongs to GT5, and a recent crystal structure showed that a SBS was present (CUESTA-SEIJO *et al.* 2013). SPR data showed that knocking out the SBS resulted in a complete loss of binding to BCD and malto-oligosaccharides.

Acknowledgement(s)

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References

COCKBURN, D. & SVENSSON, B. 2013. *Carb. Chem.* **39**: doi:10.1039/9781849737173-00204

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FUJI, K., *et al.* 2007. *J. Biosci. Bioeng.* ***103**, 167*

CUESTA-SEIJO, J.A. *et al.* (2013). *Acta Cryst. D* **69**, 1013-1025

VANDER KOI, CW. *et al.* (2010). *PNAS*, **107**, 15379-15384

Abstract No. 4

Title: Multiplexed electrical impedance-based sensing technologies for bioengineering applications

Chiara Canali[1], Arto Heiskanen[1], Ørjan Grottem Martinsen[2,3], Haseena Bashir Muhammad[1], Anders Wolff[1], Martin Dufva[1], Jenny Emnéus[1]

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[2]*Department of Physics, University of Oslo, Oslo, Norway*
[3]*Department of Biomedical and Clinical Engineering, Oslo University Hospital, Oslo Norway*

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Brief description of research area:

Adding the third dimension to the cell culture environment creates an essential need for sensing cellular faith with spatial resolution over time. Hence new combinations of more conventional bioanalytical techniques and their further improvement have become attractive challenges to overcome. Electrical impedance spectroscopy (EIS) has been successfully applied as a non-invasive and cost-effective method of measuring physico-chemical properties of biological materials depending on their composition in several fields spanning from cell biology to physiology and medical technologies. Our goal is to develop innovative EIS based platforms for noninvasive and real-time monitoring of the complex biochemical dynamics occurring during the entire process of tissue engineering from polymerization of a bare three dimensional (3D) hydrogel scaffold to stem cells encapsulation within the scaffold and cell growth and differentiation over time. Numerical simulations constitute a well-established approach in order to verify the sensitivity field distribution within an electrochemical sensor. This can be achieved by optimizing the electrode geometry, orientation and positioning within a 3D cell culture chamber and switching between different configurations of current-carrying (CC) and pick-up (PU) electrodes which together cover the entire volume of the sample under test.

What we know:

We rigorously designed and tested different innovative sensing technologies able to provide a high spatial resolution to conventional EIS by integrating the electrical impedance tomography (EIT) approach which typically shows the distribution of relative impedance changes. Each sensing platform carries an array of intelligent bioreactors (17x19x10 mm^3) for parallel analysis and may include different electrode geometries (i.e. plates and needles) which can be exploited with different sensing configurations in a multiplexed approach. This allows gathering complementary information depending on the spatial distribution of cells inside the bioreactor. The sensitivity fields simulated *in silico* were further validated with electrochemical analysis and phantom experiments. Different cell lines (mesenchymal and neural stem cells, human hepatoblastoma cells and HeLa) were encapsulated in biocompatible gelatin scaffolds and a linear correlation between increasing cell concentrations loaded and the recorded impedance was found. We monitored cell proliferation with respect to the spatial distribution of the sensitivity fields over a period of 4 days in static conditions. As end point assay gelatin scaffolds were dissolved in biocompatible conditions, cells were counted and viability was evaluated with Trypan blue assay.

What we need:

We need knowledge and techniques to:

1. deconvolute the bioimpedance values recorded with EIS to a proper image for moving towards EIT;
2. establish a protocol for cryo-slicing the scaffold without affecting cell integrity and orientation in order to correlate EIS data about cell spatial distribution with more conventional imaging methods (e.g. confocal microscopy);
3. incorporate O_2 and pH sensors in the bioreactors (in collaboration with *Luxcel Biosciences*, luxcel.com);
4. integrate fluidic pumps in order to perfuse the bioreactors with fresh cell culture medium over time;
5. scale-down the bioreactors' size and integrate them within a smaller lab-on-a-chip platform.

Abstract No. 5

Motility of muscle-fibres expressing different myosin isoforms under oxidative conditions, using an in-vitro motility assay.

D. Spanos[1], M. Li[2], C. Baron[1], L. Larsson[2]

[1]*National Food Institute, Kongens Lyngby, Denmark.* [2]*Uppsala University, Uppsala, Sweden.*

Under oxidative stress, myosin has been shown to be one of the muscle proteins that are extensively modified, leading to carbonylation and cross-linking. However, how oxidation affects the actomyosin interaction in muscle fibres with different metabolic profiles and expressing different myosin heavy chain (MyHC) isoforms has not been previously investigated. Oxidation of myosin isolated from muscle fibres originating from various porcine muscles with different metabolic profile was studied using an in-vitro motility assay. In the experimental procedure, single muscle fibres were liberated from a previously permeated fibre-bundle. The fibres were further segmented and each segment was placed within the chamber of a microscopic flow cell composed of a microscope slide and a cover slip. Infusion of a series of solutions through the chamber leads to the extraction of myosin and myosin-associated proteins, which reorganise as a high-density streak on the surface of the slide. Fluorescently-labelled actin is then added and the speed of its organised motility is quantified by the video-feed from an epifluorescence microscope. Speed measurements prior -and following- incubation with hydrogen peroxide were recorded and compared to assess the effect of myosin oxidation on motility. The metabolic profile of the single fibres was subsequently determined through SDS-PAGE, based on their MyHC isoform which was identified by their characteristic protein-band pattern on the silver-stained gel. Ultimately, the investigation evaluated the impact of an oxidative environment on the function of the main contractile protein of muscle and determined whether myosin from fibres of different metabolic profiles responds differently to oxidative stress, in regards to the in-vitro motility speed.

Abstract No. 6

Title: Evolution of *Pseudomonas aeruginosa* regulatory proteins shapes new routes of gene regulatory networks

Eva Kammer Andresen[1], Maher Abou Hachem[2], Lars Jelsbak[1].

[1] *DTU Systems Biology, Infection Microbiology Group.* [2]*DTU Systems Biology, Enzyme and Protein Chemistry.*

Brief description of research area:

Bacteria have the capacity to adapt to ecological changes by changing their gene expression profiles. During the course of evolution, gene regulatory networks may be subjected to an evolutionary shaping that allow the bacteria to breach the boundaries of already existing gene regulatory networks, create new routes of gene expression networks, and thus be able to colonize novel niches. In the Infection Microbiology Group, we use colonization of the human Cystic Fibrosis (CF) airways by the opportunistic pathogen *Pseudomonas aeruginosa* as a system for studying microbial evolution in a natural environment.

Studies of long term *P. aeruginosa* CF infections have demonstrated that evolutionary important mutations appear in genes encoding important regulatory proteins. Specifically, mutations fixed in sigma-factor proteins facilitate the generation of novel phenotypes, such as changes in antibiotic resistance, glycosylation patterns and alginate production, and completely shift the life-style of the bacterium from an environmental pathogen to a human airway specific pathogen.

We work to understand the effect of the observed mutations in sigma factors on a molecular level, as well as in connection with the entire cellular system. One approach is to study the specific protein variants *in vitro* from expressed and purified proteins, e.g. how interactions and affinities between sigma factors and DNA recognition sites are affected, and how the protein-protein interactions between known regulatory multi-protein assemblies (such as the core RNA polymerase in association with sigma-factors) are affected. A second approach is to study the gene regulatory network alterations *in vivo* to gain a complete understanding of how the entire system is affected by mutated regulatory proteins. We are using Chromatin Immunoprecipitation-sequencing (ChIP-seq) to study the *in vivo* effects of altered protein-DNA interactions, as well as overexpression of sigma factors *in vivo* to elucidate the phenotypic consequence of sigma factor competition.

These investigations will complement each other and create a better understanding of how bacteria evolve to respond to environmental stimuli such as routes of infection, development of antibiotic resistance etc.

What we know:

Microbiology, microbial interactions, microbial infections, genetic manipulations, bacterial evolution, biofilms

What we need:

In vivo techniques to study protein-protein interactions. Any interesting angles and perspectives on the subject of studying regulatory proteins in microbial infection scenarios and as pure biophysical studies.

Abstract No. 7

Title: LC-MS based differential proteomics and metabolomics in cereal research

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Brief description of research area:

Our research focus is to study cereal storage proteins (barley, wheat and rye) and hydrolytic enzyme activation by reductants and/or proteolytic activation. The workflow we adopt starts from studying the proteome of a particular set of proteins /enzymes and verify their sequences *in vitro* by peptide mapping and their function *in vivo* by 35S promoter driven overexpression and/or RNAi constructs in transgenic plants. Differential proteomics/metabolomics based on label free Progenesis LC-MS and Comet v3.0 platform is used to find candidate genes/metabolites for improving mineral or amino acid composition in selected cultivars in different growing and environmental conditions. *In vitro* validation by expressing such candidate genes as recombinant enzyme(s) in *Pichia pastoris* will further confirm their biological function.
Our target enzymes/proteins are: purple acid phosphatases, plant proteases, plant and fungal xylanases and plant xylanase inhibitors extended with the cereal reserve proteins and also metal storage proteins in the grains. On the poster a series of candidates related to zinc binding proteins of interest will be presented with an aim for future biofortification strategies in cereals.

What we know:

Today differential proteomics/metabolomics is moving to high throughput shotgun analysis of complex protein fraction without too much pre-separation ahead. Label free comparison is based on specific software which is designed to analyze 2D-SDS-Gels and now applied to detect m/s versus precise retention time (heat maps) of 1D or 2D nanoUPLC-ESI-MS1 based chromatograms. MS/MS acquisition of selected precursors is used for confirming the peptide sequence or the metabolite unique identification.
Furthermore LC-MS based "Omics" is able to detect post translational modifications and depending on the pH, metal based metallomics (metal adducts or binding omics) of peptides and metabolites.

What we need:

The use of high resolution and high mass accuracy MS instruments have evolved very rapidly from ion mobility Q-TOF MS (i.e. Waters Synapt G2-Si) to dual pressure Tribrid Orbitrap Mass Spectrometer (Thermo Scientific Orbitrap Fusion). Besides differential proteomics software like Sieve or Transomics (ex Progenesis LC-MS) are available for these difficult jobs of comparing many spectra *in silico*. Recently a new version of Comet v3.0 had been implemented to MS/MS search of metabolites in custom or commercial metabolite databases. New proteomics and metabolomics software based on algorithms that use the GPU instead of the CPU for computing are available, resulting in up to 20 x faster searches. To validate quantitatively specific peptides/compounds, the quantification is preferable by targeted approach (quantitative proteomics). The high cost of AQUA or related isotopic labeled standard peptide avoidable by the easy approach of AM/AR instruments (i.e. Q-Exactive) as targeted quantification by PRM (parallel reaction monitoring) featured in the recent Orbitrap MS instruments.
Since the Protein Research Group (part of the Department of Biochemistry & Molecular Biology at the University of Southern Denmark in Odense) represents the center for MS with emphasis on human proteomics (i.e Centre for Clinical Proteomics), it is now time for a development of a dedicated Centre for Plant Proteomics.

Abstract No. 8

Proteomic Studies of Surface, Secreted, and Membrane Proteins of *Lactobacillus acidophilus* NCFM and Effects of Potential Prebiotics on Adhesion

Hasan Ufuk Celebioglu[1], Sampo Lahtinen[2], Kristian Mølhave[3], Susanne Brix[1], Maher Abou Hachem[1], Birte Svensson[1], and Susanne Jacobsen[1]

[1]Departmnet of Systems Biology, DTU. [2]DuPont, Health & Nutrition. [3]Department of Micro- and Nanotechnology Nanotech, DTU.

Brief description of research area:

Adhesion of probiotics to the gastrointestinal tract is considered to be an important criterion for colonization. *Lactobacillus acidophilus* NCFM (NCFM) is one of the well-defined probiotic strains isolated from humans and used in dietary supplements and yogurts. Although the adhesion process is complex and involves a variety of proteins, Surface layer (S-layer) and secreted proteins have been found to have roles in this adhesion. Combination of probiotics with emerging prebiotics has been shown to alter protein expression in a way that might change the functional properties of the probiotic.

What we know:

We are experienced in 2-Dimensional Gel Electrophoresis, Mass Spectrometry, cell culture, as well as adhesion assays for NCFM to mucus and human intestinal HT-29 cells. In addition, we are collaborating with DTU Nanotech for Scanning Electron Microscopy to visualize the interactions between NCFM and HT-29 cells.

What we need:

Although Liquid Chromatography-coupled Mass Spectrometry (LC-MS) is used for membrane proteomics, we have to optimize the extraction of membrane proteins and sample preparation for LC-MS. Moreover, we are seeking techniques to determine the interactions between secreted or surface-layer proteins from probiotics and glycoprotein mucin, which is the protein component of mucus.

Abstract No. 9

Abstract template

Title: Title: Altering the activation mechanism of *Thermomyces lanuginosus* lipase

Jakob Skjold-Jørgensen[1,2], Jesper Vind[2], Allan Svendsen[2], Morten J. Bjerrum[1]

[1]University of Copenhagen, [2]Novozymes

Brief description of research area:

The aim of this project is to modify the activation mechanism in a lipase from the thermophilic fungus, *Thermomyces lanuginosus* lipase, by rational design of the amino acid residues located in a structural domain, called the "lid", covering the active site. The research embraces protein engineering, molecular biology, protein purification and protein characterization.

What we know:

We have expertise in the generation of lipase variants, MD simulations, protein purification using hydrophobic interaction and anion exchange chromatography. We apply different MTP scale activity, stability and fluorescence based assays. Site-directed-fluorescent-labeling (SDFL) is used to create variants suited for lid-dynamic investigations *in vitro*. Steady state fluorescence spectroscopy is used.

What we need:

We need to develop a fluorescence based technique that enables the measurement of small secondary structural movements (activation) of lipases *in vitro* e.g. time-resolved, single molecule fluorescence spectroscopy.

We need to strengthen understanding of the parameters that are most dominant in determining lipase activation using other techniques e.g. NMR etc.

How can protein dynamics be measured for movements in the 0-20Å range, *in vitro*?

We need good model-micelles for studying lipase activation in fluorescence based assays.

Abstract No. 10

Title: HEXPIN: Hetero-exopolysaccharide – milk protein interactions.

Johnny Birch[1], Maher Abou Hachem[1], Per Hägglund[1], Richard Ipsen[2], Kristoffer Almdal[3], Günther H. Peters[4], Pernille Harris[4], Christel Garrigues[5], Birte Svensson[1]

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[5] CED-Discovery, Chr Hansen A/S, Bøge Allé 10-12, 2970 Hørsholm, Denmark

Brief description of research area:

Polysaccharides from plants or seaweeds, often chemically modified, are extensively used as biothickeners by today's food industry. Their use is however, strongly restricted and food products enriched with these need to be labeled with E-numbers. Numerous studies have established that hetero-exopolysaccharides (HePS) excreted by various lactic acid bacteria (LAB) strains have a positive impact on textural properties in fermented milk products. HePS are natural ingredients and *generally recognized as safe* (GRAS), and thus *in situ* production of HePS needs no declaration. The textural effect in fermented milk products is due to the extent of complexation between HePS and milk proteins. However, the correlation at the molecular level between HePS-milk proteins interactions and the textural properties is poorly understood.

The aim of this project is to screen for complexation between structurally characterized HePS and milk proteins by surface plasmon resonance (SPR), a method also proven suitable for analyzing binding constants and rates of oligosaccharide-protein interactions [1,2]. HePS-milk proteins interactions will be further characterized by various biophysical, micro- and macroscopic analyses. Additionally, HePS repeat units will be prepared to understand the contribution of individual structural elements in the protein binding. Ultimately, identification of structural elements important for HePS-milk protein complex formation might lead to the ability to selectively choose specific HePS to achieve desirable textural properties without the use of additives. Recently, we have established an electronic database based on the structural and biophysical properties of LAB HePS found in the literature.

What we know:

- Homo-exopolysaccharides (HoPS) composed of various linear and branched Ά-glucans has no significant impact on formation of the textural matrix.
- HePS, which are composed of different monosaccharides e.g. galactose, glucose, rhamnose, and N-acetyl-galactosamine, contribute vitally to texture and quality of yoghurt and cheese.
- Interaction between HoPS and milk proteins (beta-lactoglobulin and kappa-casein) are highly specific and dependent on molecular weight, linkage type and degree of branching of the HoPS.

What we need:

- To produce known HePS structure by fermentation of lactic acid bacteria strains.
- To identify smart ways of producing HePS oligosaccharide repeats.

Acknowledgment:
The project is funded by the Danish Research Council for Independent Research | Technical and Production Sciences.

References:

1 Nielsen, M. M. *et al.*, (2009). Biochemistry **48**, 7686–97.

2 Diemer, S. K. *et al.*, (2012). Food Biophys **7**, 220–226.

Abstract No. 11

Structural Studies of Protein-Protein Interactions: FLRT Interactions with the FGF Receptor

Lila Yang[1], Kim Krighaar Rasmussen[2,1], Maria Hansen[2], Peter Schledermann Walmod[2], Leila Lo Leggio[1]

1- Department of Chemistry, Faculty of Science, University of Copenhagen

2- Department of Neuroscience and Pharmacology, Faculty of Health Sciences, University of Copenhagen

Brief description of research area

Protein-protein interactions are key in most biological processes and hence also in disease states. Fibroblast Growth Factor Receptors (FGFRs) are implicated in a number of disease states and are trans-membrane receptors whose extracellular region comprises three modules--Ig1, Ig 2 and Ig3. Fibronectin Leucine Rich Transmembrane (FLRT) Proteins consist of extracellular leucine-rich repeats (LRRs), a fibronectin type III (Fn III) domain and a cytoplasmic tail. FLRT3 is the best-characterized member of the FLRT family, has been shown to participate in the Fibroblast Growth Factor signaling pathway.

What we know

We cloned, expressed and purified FnIII of FLRT isoforms 1, 2 and 3. Pure FnIII of FLRT1 and 3 have been obtained and their CD spectroscopy and NMR showing predominantly secondary structures have been performed. Published studies have indicated that the FnIII domain of FLRT3 mediates interaction between FLRT3 and FGFRs. At the Department of Neuroscience, tetravalent peptide ligands derived from the human FLRT3-FnIII sequence have shown submicromolar affinities for FGFR. Initial SPR also reveals FLRT3 binds to FGFR construct containing the Ig2-Ig3 modules.

What we need

We will try to characterize the interaction of the FnIII domain of FLRT3 and Ig2-Ig3 of FGFR by X-ray crystallography and small angle X-ray scattering (SAXS), for which we already have expertise. We would like to come in contact with groups who are expressing fully deuterated proteins in *E. coli* as this would aid us in neutron studies.

Abstract No. 12

Design of proteinaceous inhibitors of industrially important starch degrading enzymes and proteases

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Brief description of research area:
Proteinaceous enzyme inhibitors have been shown to not only regulate the activity, but also stabilise their target enzymes. The project focuses on design of inhibitors of two groups of industrial enzymes, starch hydrolases and serine-proteases. Prolonging shelf-life and regulation of enzymes are important goals. Today chemicals are used, e.g. for reducing autoproteolysis of proteases. Inhibitors may also be used for recycling enzymes from industrial processes. A biochemically and structurally characterised limit dextrinase inhibitor (LDI) [1] will serve as template for computational protein design. LDI is from a family of α-amylase and serine-protease inhibitors, and we have evidence for interaction hotspots in the complex with its target enzyme. The combination of advanced computational methods and experimental characterisation of inhibitor variants offers a strong approach to achieve useful potent enzyme inhibitors.

What we know:
Computational methods for protein design/redesign involving the ROSETTA molecular modelling suite; recombinant protein production in *Pichia pastoris* by high cell density fermentation; protein structure determination by X-ray crystallography; analysis of protein-protein interaction e.g. by surface plasmon resonance (SPR).

What we need:
Knowledge about design of the experimental setup for optimisation of protein binders by the combination of mutagenesis and yeast surface display

This work is supported by The Danish Council for Independent Research|Technology and Production Sciences, a Sapere Aude-Research Talent grant from the Danish Council for Independent Research (to MSM).

[1] Møller, M.S., Vester-Christensen, M.B., Jensen, J.M., Abou Hachem, M., Henriksen, A., and Svensson, B.. Structure of the complex between barley limit dextrinase and its endogenous inhibitor sheds light on the functional versatility of cereal-type inhibitors. (In preparation)

Abstract No. 13

Title: Designing knowledge based potentials with near-native protein funnels by semi-definite optimization

Martin Carlsen[1], Peter Røgen[1]

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Brief description of research area:

Application of mathematical methods such as linear algebra, convex optimization, b-splines and metrics for protein structure prediction.

What we know:

We are primarily concerned with the modeling of protein energy landscapes to be used for refinement of near-native protein structures. We use an indefinite Hessian-based optimization method for refinement of individual protein models and we design protein potentials with local minima in the energy landscape in a number of native structures using a semi-definite optimization strategy. Our potentials are spanned by b-splines and trained on native-decoy distances.

What we need:

- A more profound understanding of why it is difficult to refine near-native structures and why force fields such as the CHARMM force fields fails.
- An understanding of how many energy terms are sufficient to stabilize a protein. Currently, we use a backbone potential consisting of a pair potential, a local potential, a solvent potential and a hydrogen bond potential.
- We design a local basin of attraction by requiring that the potential has a vanishing gradient and a positive semi-definite Hessian for a set of native structures. What would it require to ensure dynamic stability?

Abstract No. 14

Title: Protein carbonylation in bacteria and disease.

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Brief description of research area:

Protein carbonylation has been linked to a myriad of diseases as well as cell senescence. Carbonylation is specific, and depends on the oxidative agent which makes it a possible target for biomarker development.

My research consists of development and optimization of a method for identification of specific carbonylation sites in proteins. Detection requires a highly sensitive detection method as carbonyls are low in abundance. To maximize sensitivity, biotin/avidin enrichment on the peptide level is coupled with mass spectrometry.

The next step will be identification of carbonylation patterns in oxidatively stressed bacteria and diseased individuals, for industrial or medicinal use.

What we know:

Protein carbonylation has historically been used as an indicator of oxidative stress through different quantitative methods, mostly utilizing aldehyde/ketone specific reagents coupled with an indicator such as a fluorophore. These methods were used to show correlation between carbonylation and several diseases, and constitute a basis from which current methods develop.

In contrast to reversible oxidative modifications, such as cysteine disulfides or methionine sulfoxides, formation of protein carbonyls cannot be reversed by cell repair machineries. Carbonylated proteins are typically targeted for proteasomal degradation or they might accumulate in supramolecular aggregates, as seen in Alzheimer's disease and Parkinson's disease. In addition to development of biomarkers, protein carbonylation might correlate a disease with a specific type of oxidative stress through the carbonylation fingerprint.

To further the understanding of carbonylation, different techniques have been developed that attempt to identify the specific sites. One of the more promising methods utilizes biotin/avidin enrichment and mass spectrometry. This method has been used to shed some light on the specificity of carbonyls induced by metal catalyzed oxidation, and also shown a specific carbonylation pattern in serum samples of breast cancer patients. Further development of this method seems to have improved the output, though more samples have to be processed before a conclusion can be made.

What we need:

Method development: Specifically in peptide purification.

Data handling: As more samples are run the data sets get too large for manual inspection and have to be handled by scripts in Matlab, R, (Possibly excel).

Abstract No. 15

Title: Development of a recombinant antibody-based treatment of snakebites

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[1]*Department of Systems Biology, Technical University of Denmark*

Brief description of research area:

Venomous snakebites are regarded as one of the World's most neglected tropical diseases with more than 5 million victims every year. The only current treatment option is low quality antivenom with severe adverse reactions. These are so expensive that the life- and limb saving treatment is often not employed.

This project aims at replacing existing snake antivenoms produced by immunization of large mammals with snake venom with recombinant, humanized antibodies produced by modern cell-based fermentation technology. It is anticipated that such an antivenom will reduce the current high risk of severe side effects, reduce cost, and make essential antivenom available for the more than 5 million snakebite victims every year. Cheaper and safer antivenoms will lead to reduced mortality and a reduction in the number of victims suffering from permanent disabilities – particularly in low- and middle-income countries in the tropical and sub-tropical areas of the World.

Modern day technology allows development of monoclonal antibodies (mAbs) targeting snake toxins, however, identification, characterization of immunogenic features (B-cell epitopes), and availability of snake toxins currently constitutes a major bottleneck blocking the development of recombinant mAbs. We will set out to remove this bottleneck by identification of snake toxins based on transcriptome analysis, identification of antibody binding sites in toxins using peptide arrays, recombinant expression, and characterization of toxic snake proteins and subsequent generation of an mAb targeting snake toxins resulting in a proof-of-concept mAb-based antivenom.

This project focuses on the serious threat caused by bites of one of the four mamba snake species (Dendroaspis) in South-, Eastern-, and Central Africa. The project will entail expression of several neurotoxins and lab scale production of mAbs targeting common features of mamba toxins using mammalian cell factories.

What we know:

We are currently investigating shared surface patterns and epitopes on mamba toxins using a combination of protein modeling, peptide microarray and phage display. We have identified key residues responsible for toxicity of important neurotoxins and will soon be ready to express toxins as well as our (ideally) non-toxic analogs.

What we need:

We will soon need protein analysis methods and skills. One example of an issue to be addressed is how to validate the correct fold / disulfide bridge structure of our expressed toxins and analog.

Abstract No. 16

Oligosaccharide uptake in *Bifidobacterium animalis* subsp. *lactis*

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Glycan utilization plays a key role in modulating the composition of the gut microbiota. Here we demonstrate how biochemical characterization of the carbohydrate transport capabilities of gut bacteria can be used to determine the preferred carbohydrate substrates of probiotic (health promoting) bacteria, which is relevant in symbiotic formulations of to develop more potent probiotic based food additives. This also furthers our understanding of the metabolic specializations of different gut bacteria.

Bifidobacterium animalis subsp. *lactis* Bl-04 is a widely used probiotic bacterium from the *Bifidobacterium* genus that have adapted to the habitat of the human gastrointestinal tract. *B. animalis* subsp. *lactis* Bl-04 possesses a very limited number of secreted glycoside hydrolases, to utilize undigested glycans e.g. resistant starches and plant cell wall polysaccharides as carbon and energy source. Instead this organism act as a secondary degrader that depends on cross-feeding from other taxa *e.g. Bacteriodes* and *Roseburia* spp., which perform the primary degradation of the above mentioned glycans. Efficient oligosaccharides import systems are instrumental in this highly competitive lifestyle. *Bifidobacteria animalis* subsp. *lactis* Bl-04 posseses, 5 ABC-type oligosaccharides importers. We have characterized two ABC-transporter associated solute binding proteins (SBP) targeting arabinoxyloolgosaccharides and soybean family oligosaccharides, which are two diet abundant prebiotic carbohydrate types. The structures, binding kinetics and energetics of these binding proteins give a view into the evolution of bifidobacterial transport systems in adaption to the competitive and dynamic gut niche.

Abstract No. 17

Purification and inactivation of Clostridium difficile Toxins A and B

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Brief description of research area:

Clostridium difficile toxins A and B are exotoxins responsible for a medical condition known as pseudomembranous colitis (PMC). PMC affects both humans and animals, and cause symptoms of diarrhea brought on by antibiotic use. Toxin A and B are both glucosyltransferases which target and inactivate certain Rho GTPase proteins. The inactivation of Rho, Rac and Cdc42, by toxins A and B affects actin formation, and induces morphological changes such as cell rounding, and apoptosis.

Our aim is to develop a protocol for inactivation of structurally intact toxin A and B with the intention producing a toxoid-based vaccine against infections by *C. difficile*.

The toxins are both expressed, when cultured anaerobically using dialysis, and have a molecular weight of 308 kDa (Toxin A) and 270 kDa (Toxin B), respectively. Toxins A and B have been purified using anion exchange chromatography and gel filtration. The purified toxins were tested for cytotoxic activity by adding dilutions of the toxin to Hela cells and evaluating the morphological changes to the cell. As an alternative to formaldehyde inactivated toxoids we intend to use H_2O_2 for inactivating the proteins.

What we know:

Protein purification (Dialysis), Anion Exchange Chromatography, Cytotoxicity Assay

What we need:

Protein Inactivation, Vaccine Testing

Abstract No. 18

Title: Characterization of the thioredoxin system in *Lactococcus lactis*

Nicklas Skjoldager[1], Petr Efler, Martin Rykær[1], Olof Björnberg, Susanne Jacobsen[1], Birte Svensson[2], Per Hägglund[1].

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Brief description of research area:

Lactococcus lactis is a Gram-positive bacterium, which is commonly used as a starter culture in production of cheese, butter milk and wine.

In the presence of oxygen it is inevitable that there will be a cellular oxidative stress in the form of reactive oxidative species (ROS) like H_2O_2, O_2^- and $OH^\cdot$. This can lead to damage of the DNA, RNA, proteins, lipids ect.

In order to cope with oxidative stress *L. lactis* is equipped with a thioredoxin disulfide reduction system that transfer reducing equivalent from NADPH-dependent thioredoxin reductase (TrxB) to two thioredoxins (TrxA and TrxD), with in turn can reduce disulfide bonds in target proteins.

TrxA is a classical thioredoxin with a conserved WCGPC motif in the active site. TrxD on the other hand is an atypical thioredoxin with a WCGDC motif and mainly reported from bacteria in the phylum of *Firmicutes*.

What we know:

The enzymes TrxB, TrxA and TrxD have been expressed and tested in kinetic assays with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as the final electron acceptor. Both TrxA and TrxD are recycled by *L. lactis* TrxB. TrxR from *E. coli*, on the other hand, can recycle TrxA but *not* TrxD. Surprisingly, TrxD cannot reduce insulin, which is classically a standard substrate for testing thioredoxiner. Deletion mutants of *ΔtrxA*, *ΔtrxD* and *ΔtrxAΔtrxD* are available. Under oxidative stress *ΔtrxD* grows almost like the wild type, while *ΔtrxA* has decreased growth rate and the double mutant is critically impacted. Arsenate and tellurite sensitivity is the only phenotype detected of the *ΔtrxD* mutant. Potential TrxD targets have been determined *in vitro* with 2-D fluorescence Gel Electrophoresis, and subsequent MALDI-TOF MS for protein identification. Arsenate reductase and selected TrxA/TrxD targets are now being expressed recombinantly for elucidating the functions of TrxA and TrxD

What we need:

To understand the mechanistic functions of TrxA and TrxD.

The physiological function of TrxA and TrxD.

Target proteins for TrxA and TrxD.

Abstract No. 19

Title: Activity-Based Protein Probes for Investigation of Lysine Acylation

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Brief description of research area: The field of epigenetics has fascinated the scientific community in recent years. More specifically, lysine ε-amino acetylation has been long recognized as an epigenetically important post-translational modification. Our research is focused on development of tools to characterize acyl-based post-translational modification of lysine. We synthesized tri-functional activity based probes, the binding of which were tested against histone deacetylases and sirtuins. Our experimental approach for the project and the key results will be discussed.

What we know: Our laboratory is well equipped to perform research on peptides with our state-of-the-art organic synthesis and solid phase synthesis expertise, analytical and preparative scale high-performance liquid chromatography equipment and fluorometric assay screens. We are proficient in synthesis of functionalized peptides and macro-cyclic peptides in addition to performing fluorescence based enzyme kinetics. Further, we are in a process to develop a well-equipped molecular biology section, wherein we already possess the basic facilities like that of SDS gel electrophoresis and western blot module.

What we need: We are planning on expanding our research work using several molecular biology techniques and methodologies for example protein production and cell based studies. In this respect, we would like to get in touch with groups who are already well equipped with protein research tools. In particular, we would be interested to know about the available tools for protein detection, one of them being fluorescent scanners for SDS gels.

Abstract No. 20

An insect-based model for drug permeability screening across the blood brain barrier: Transcriptomic and functional analysis

Sonia Al-qadi[1*], Morten Schiøtt[2], Steen H Hansen[1], Peter A Nielsen[3] and Lassina Badolo[4].

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Grasshopper (locust) has recently been developed as a time- and cost-effective insect-based model, for permeability screening of drug candidates across the human blood brain barrier (BBB) *ex vivo*. This model affords technical convenience and ethical acceptability and is expected to show high reproducibility, predictability as well as high throughput drug screening. It is widely recognized that ABC efflux transporters, namely the p-glycoprotein (p-gp) encoded by the ABCB1 gene, play a major role in drug pharmacokinetics at the BBB. Therefore, the identification of such transporters in locust brain will certainly render this model reliable for drug screening. Accordingly, the aim of the current project is to perform transcriptomic profiling of putative ABC efflux proteins in locust brain, in addition to functional characterizations. Lastly, comparative gene and functional studies will be performed with other vertebrate BBB models and *in vitro* cell systems, in order to elucidate the prediction power of locust under development as well as its validity and utility for permeability testing in the early phase of drug discovery.

What we know:

Analysis of drug transport kinetics across the BBB *ex vivo* as well as transcriptomic and bioinformatic processing

What we need:

Immunohistochemical examination of the most important molecular components of efflux activity, like the human p-gp ortholog of locust, in order to identify its subcellular localization in the locust brain. So, we need an antibody that can selectively target this protein for accurate detection.

Abstract No. 21

Structural studies of β(1,6)-galactosidase from *Bifidobacterium animalis* subsp. *lactis* Bl-04 from the GH42 family in complex with small ligands

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Brief description of research area:

*Bl*Gal42A is a β-galactosidase expressed in the probiotic *Bifidobacterium animalis* subsp. *lactis* found in the human gastrointestinal tract. Understanding the structure-function relationship of substrate binding in β-galactosidases from different probiotic organisms can help us understand how these organisms adapt to very specific ecological niches. In order to do so, we are using X-ray crystallography and different approaches to study the interaction of BlGal42A with ligands.

What we know:

The structure of *Bl*Gal42A has been solved (Viborg *et al.*, unpublished) and we have worked on the basis of this structure to investigate structures of both native and active site-mutated enzymes (*Bl*Gal42A Glu324Ala), to gain understanding of the active site. Here we will present the structure of the active site mutant in complex with an α-D-galactose and conformational changes upon binding in the active site. We will present our progress in obtaining crystal complexes with small galactose-derived inhibitors as well.

What we need:

Despite several attempts to crystallize the inactive mutant with oligosaccharides, we have not yet succeeded. We are very interested in potential oligosaccharide-like inhibitors to carry out similar studies with the native enzyme.

Abstract No. 22

Biochemical characterisation of a GH62 α-L-arabinofuranosidase from *Aspergillus nidulans*

Susan Andersen[1], Casper Wilkens[1], Silvia V. Melgosa[2], Darrell Cockburn[1], Henriette L. Petersen[2], Hiroyuki Nakai[1], Hans E. M. Christensen[3], William G. T. Willats[2], Maher Abou Hachem[1] and Birte Svensson[1]

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Brief description of research area:

Arabinosyl residues are abundant in plant cell wall matrices, both as arabinan present in pectin and in hemicelluloses as side chains in arabinoxylans. The removal of the arabinosyl residues is important in the degradation of plant material in bioethanol production and other industrial processes (Numan & Bhosle, 2006). Furthermore, arabinose residues are shown to be useful in control of the blood sugar level in diabetics (Inoue *et al.*, 2000).

Glycoside hydrolase family 62 (GH62), which only comprise α-1,5-L-arabinofuranosidases (ABF), has received little attention and the current biochemical data are deficient. Activity was reported toward xylans, arabinoxylans (Vincent *et al.*, 1997), arabinogalactans and arabinans (Kimura *et al.*, 2000). Kinetics, however, on natural substrates are only reported for one enzyme (Vincent *et al.*, 1997). Neither the catalytic residues nor the catalytic mechanism have been determined experimentally. GH62 is related to GH43, a relatively well characterized family comprising several specificities, and together they form the GH-F clan in the CAZy database (Cantarel *et al.*, 2009). It is therefore likely that the two families share mechanism and certain enzymatic properties.

What we know:

GH62 ABF from *Aspergillus nidulans* FGSC (*An*GH62) was found to interact with wheat arabinoxylan, xylans and barley β-glucan in affinity gel electrophoresis (AE), but not with sugar beet L-arabinan, whereas enzymatic activity was detected on wheat arabinoxylan and sugar beet L-arabinan. Furthermore, enzymatic activity was detected on arabinoxylan oligosaccharides and *para*-nitrophenyl-α-L-arabinofuranoside. Based on alignments and kinetic data we found three important catalytic residues, which correspond to the general acid and base, and the pK_a modulator found in GH43.

What we need: Arabinoxylan and arabinan oligosaccharides.

Abstract No. 23

Title: Amino acid analysis, protein sequencing and mass spectrometry

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Enzyme and Protein Chemistry, Department of Systems Biology, building 224, DTU, hosts the protein core facility – for more detailed information and guidelines: see link http://www.bio.dtu.dk/english/Research/Research-groups/Enzyme-and-Protein-Chemistry/Services

The protein core facility offers:

- Amino acid analysis
  Amount
  Composition and identification
- Mass spectrometry
  Identifications of proteins
- Protein identification
  Identification of proteins
  Homology search
  Information of post-translational cleavage
  Information about purity

The services of the core facility are available for researchers and students at DTU, other academia and commercial customers.

For more practical information:

You are welcome to contact us at the Protein.DTU workshop.

Abstract No. 24

Novel biomarkers for change in muscle mass or muscle pathology

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Brief description of research area: Focusing in different diseases, the collaboration between DTU and Nordic Bioscience aims at developing biomarkers used in ELISA assays. Having success in bone, liver, cancer, metabolic and Alzheimer's disease, a recent frame expansion towards muscle tissue is the latest step. Muscle is being degenerated and reconstructed as part of basic metabolism but certain pathologies lead to severe loss of muscle function and mass. This project is in particular involved in identifying novel biomarkers that will be used for prognosis or diagnosis of muscle dystrophies or myopathies.

What we know: There is a notable previous success in identifying candidate biomarkers in bodily fluids as well as raising antibodies against said protein biomarkers. In particular, interest lies in the neoepitopes, a term coined to describe a site of post-translational modification of a protein that is specific to the disease under investigation.

What we need: Part of the project will be an LC-MS prospective analysis held in two study groups (patient's samples and healthy controls) to map differences in the blood proteome or tissue samples. Expertise in pretreatment of samples, ideally avoiding use of proteases or extensive pre-process of the specimens, is required. Furthermore culturing a cell based model system, representative of the muscle tissue would be of interest.

Abstract No. 25

Title: Decoding Nitrosylation in Bacteria and Humans

Zacharias Brimnes Visby Damholt\ Susanne Jacobsen[1], Birte Svensson[2], Anne-Christine Bay-Jensen3 and Per Hagglund[1]

[1]*Protein and Immune Systems Biology* [2]*Enzyme and Protein Chemistry* [3]*Nordic Bioscience*

Brief description of research area:
Many cellular functions and pathways are regulated via posttranslational modifications of proteins. These protein modifications can result in a variety of outcomes including changing the activity, function and location ofthe protein and hence cause critical perturbations to the cellular state. Modification of protein cysteine residues with nitric oxide (NO) is one of the modifications that in recent years have gained a lot of attention. By a process called S-nitrosylation the nitric oxide reacts with the thiol group of a cysteine residue, resulting in the formation of a nitroso group on the cysteine.
It has become evident that failure in the regulation of protein S-nitrosylation is the cause of a broad range of diseases including inflammatory diseases such as fibrotic pulmonary disorders and rheumatoid arthritis, neurodegenerative disorders and cancer.
The overall aim of this PhD study is to use mass spectrometry-based proteomics to identify and characterise S-nitrosylated proteins and peptides and hereby:

1) Increase the understanding of mechanisms of oxidative stress and nitrosylation resistance in bacteria.

2) Identify S-nitrosylated proteins or peptides that reflect specific oxidative and inflammatory processes with specific emphasis on human cell and tissue models involved in inflammatory diseases such as fibrotic pulmonary disorders and rheumatoid arthritis.

3) Identify and develop biomarkers of S-nitrosylated proteins or peptides from the above mentioned diseases.

4) Investigate the presence of nitrosylated proteins/peptides in urine and tissue samples from patients with the mentioned disorders through collaboration with hospitals in the Copenhagen area.

What we know:
Mass spectrometry based proteomics, Q-Exactive. Data interpretat on of ass spec results. Ana!ys1s of post-translational modifications. Investigating novel and unknown mteract1on partners to protems.

What we need:
Bioinformatics regarding large scale data handling, gene annotations, and structural site annotations of proteomics data. Follow up via standard techniques in microbiology.

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