10th PROTEIN.DTU Workshop

27th November 2013

Contents

DTU Systems Biology

10th Workshop in Protein.DTU

27th November 2013
Building 116, Lecture Hall 81

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

Chair: Birte Svensson (Professor, DTU Systems Biology)

09:00 - 09:10 **Henrik C. Wegener** (Provost, Technical University of Denmark)
Welcome

Chair: (Jenny Emnéus, Professor, DTU Nanotech)

09:10 - 09:40 **Luc Brunsveld** (Professor, Chemical Biology, Department of Biochemical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands)
(Supra)molecular control over protein dimerization and assembly; from fluorescent proteins to caspases and nuclear receptors

Chair: Pernille Harris (Associate Professor, DTU Chemistry)

09:40 – 10:10 **Anne Houdusse-Juille** (Research Director at CNRS, Structural Motility Group, Institut Curie, Paris, France)
Allostery is at the heart of force production-Structural insights from myosin nanomotors

Chair: Michael Engelbrecht Nielsen (Associate Professor, DTU Food)

10:10 – 10:40 **Karola Schlinkmann** (Research Associate, Plückthun Group, Department of Biochemistry, University of Zurich, Switzerland)
Maximizing expression and detergent stability of a transmembrane G protein-coupled receptor by exhaustive recombination and evolution

10:40 – 11:05 **Coffee Break**

Chair: Christine Finnie (Associate Professor, DTU Systems Biology)

11:05 – 11:35 **Kenji Maeda** (Postdoctoral Fellow, Gavin Group, European Molecular Biology Laboratory, Heidelberg, Germany)
Interactome map uncovers phosphatidylserine transport by oxysterol-binding proteins

Chair: Birte Svensson (Professor, DTU Systems Biology)

11:35 – 12:15 **Sir John Walker** (Professor, FRS, FMed Sci, MRC Mitochondrial Biology Unit, Cambridge, United Kingdom)
The mitochondrial proteome

12:15 – 12:45 **Lunch and poster viewing**

Chairs: Kristoffer Almdal, Jenny Emnéus, Peter Heegaard

12:45 – 13:30 **Poster Session, coffee, drinks and cake**

Chair: Günther H.J. Peters (Associate Professor, DTU Chemistry)

13:30 – 14:00 **Birte Höcker** (Group Leader, Protein Design Group, Max Planck Institute for Developmental Biology, Tübingen, Germany)

Design of protein structure and function

Chair: Pernille Harris (Associate Professor, DTU Chemistry)

14:00 – 14:30 **Richard Neutze** (Professor, Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden)

Serial femtosecond crystallography & time-resolved studies at X-ray free electron lasers

Chair: Flemming Jessen (Senior Scientist, DTU Food)

14:30 – 15:00 **Fredrik Levander** (Senior Lecturer, Department of Immunotechnology, Lund University, Lund, Sweden)

Turning the black box of quantitative proteomics grey

15:00 – 15:20 **Coffee Break**

Chair: Kristoffer Almdal (Professor, DTU Nanotech)

15:20 – 15:50 **Matthias Rief** (Professor, Department of Physics, Technical University of Munich, Garching, Germany)

Single molecule mechanics of proteins

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

15:50 – 16:20 **Bernard Henrissat** (Research Director at CNRS, Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille University, Marseille, France)

Carbohydrate-active enzymes in the (meta)genomic era

16:20 – 17:30 **Closing and "time for a beer" and posters**

List of abstracts

Abstract no

1 Alexander Striebeck: Yeast Mnn9 is Both a Priming Glycosyltransferase and an Allosteric Activator of Mannan Biosynthesis

2 Alexander Holm Viborg: Structure-function relationship of glycoside hydrolase family 42-β-galactosidases important for prebiotic metabolism in probiotics

3 Andrea Casci Ceccacci: How Carbon Nanostructures Affect Differentiation of Dopaminergic Neutral Stem Cell

4 Bala Krishna Prabhala: New Insights Into the Substrate Specificities of Proton-coupled Oligopeptide Transporters from *E. coli* by a pH Sensitive Assay

5 Birgitte Munch-Petersen: Tomato thymidine kinase is under poor TTP feed-back regulation

6 Cecilia Emanuelsson: DNAJB6 is a peptide-binding chaperone which can suppress amyloid fibrillation of polyglutamine peptides at substoichiometric moloar ratios

7 Chiara Canali: Bioimpedance based Lab-on-a-Chip (LOC) systems for tissue engineering

8 Chris Juul Hedegaard: Swine plasma immunoglobulins for prevention and treatment of post-weaning diarrhoea: Optimizing stability towards gut conditions

9 Christian Ruzanski: Structural and Enzymatic Charaterization of Starch Phosphorylase from Barley

10 Elizabeth Wood: Phosphate and Arsenate Binding in P-Loops

11 Guiseppe Dionisio: LC-MS based differential proteomics as tool for high throughput assessing differences in plant and microbial research

12 Heike Rösner: Residual Structure in Unfolded Proteins

13 Husam Mohammad Ali Alsarraf: Structural, functional and kinetic characterization of Lympostatin, a potential glycosyltransferase toxin from *E. coli*

14 Johnny Birch: HEXPIN: Hetero-exopolysaccharide-milk protein interactions

15 Jose A. Cuesta-Seijo: Structure of CLG1_GBBS, a starch synthase from a cyanobacterium

16 Julie Bomholt: Recombinant Production of Human Aquaporin-1 to an Exceptional High Membrane Density in *Saccharomyces cerevisiae*

17 Katarzyna Krucewicz: Detailed Time Resolved Immunologic Characterization of Starch Active Enzymes throughout Barley Endosperm Development

18 Katsiaryna Skryhan: Redox regulation of starch biosynthetic enzymes in *Arabidopsis thaliana*

19 Kristian Frandsen: Binding of the N-terminal domain of the lactococcal bacteriophage TP901-1 CI repressor to its target DNA: a crystallography, small angle scattering and NMR study

20 Laura Nekiunaite: Exploring the Secretomes of Starch and Chitin Degrading Fungi

21 Louise Bundgaard: SRM and QconCAT based analysis of cytokines, acute phase proteins and hypoxia related proteins in the horse

22 Louise Helene Søgaard Jensen: Charaterizing mikro- and nanostructures in soft and wet samples

23 Louise F. Nikolajsen: The membrane proximal region of the intracellular part of the human growth hormone receptor is intrinsically disordered

24 Mamoru Nishimoto: One-pot enzymatic production of GalNAc from GlcNAc

25 Maria Grishkova: Features of oligopeptidase B from Serratia proteamaculans

List of abstracts

Abstract no 1

Yeast Mnn9 is Both a Priming Glycosyltransferase and an Allosteric Activator of Mannan Biosynthesis

Alexander Striebeck[1,2], Alexander W. Schuettelkopf[1,3] and Daan M.F. van Aalten[1]

[1]Division of Molecular Microbiology, College of Life Sciences, University of Dundee, DD1 5EH, Scotland, UK
[2]Present address: Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-1799 Copenhagen V, Denmark
[3]Present address: Structural Biology Group, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Glasgow, G61 1BD, Scotland, UK

Brief description of research area:

Our research is focused on the glycosylation of mannoproteins which form the outer layer of the fungal cell wall: mannan. Weakening mannan leads to aberrant cell walls and a higher susceptibility to antifungal drugs. We recombinantly expressed and purified two key glycosyltransferases of mannoprotein biosynthesis, Mnn9 and Van1, and solved the protein structure of Mnn9. We also identified that not only the product, but the presence of Mnn9 is necessary for activity of Van1.

What we know:

Our field of interest covers glycosyltransferases and –hydrolases. We know the essential steps to produce recombinant protein, including finding optimal gene constructs, molecular biology, expression of gene of interests including many different affinity-tags (e.g. His, GST, MBP) as well as protein crystallization and structure determination. Furthermore we develop novel enzyme assays and, if accessible, determine in vivo effects of mutations in our proteins of interest.

What we need:

After recently moved to Monica Palcic's group at the Carlsberg Laboratory I am now interested in the expression of plant glycosyl hydrolases. The aim is to understand the interaction of two isoamylases from barley. At the moment expression is good, but solubility is non-existent. I would like to gain more knowledge about opportunities to improve solubility, such has high-throughput screening of gene constructs, growth conditions and lysis buffers or about expression systems besides *E. coli* and *P. pastoris*. In particular, plant-based expression systems.

Abstract no 2

Structure-function relationship of glycoside hydrolase family 42 β-galactosidases important for prebiotic metabolism in probiotics

Alexander Holm Viborg[1]; Maher Abou Hachem[1]; Folmer Fredslund[2]; Takane Katayama[3]; Motomitsu Kitaoka[4], Leila Lo Leggio[5]; Birte Svensson[1]

[1]Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark. [2]MAX IV Laboratory, Lund University. [3]Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Japan. [4]National Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Japan. [5]Biophysical and Bioinorganic Chemistry Group, Department of Chemistry, University of Copenhagen.

Brief description of research area:

Probiotics is a part of the gastrointestinal microbiota, and are believed to play an important role in maintaining and promoting human health. Probiotics utilize a diverse range of carbohydrates which are oligosaccharides derived from milk and plants that escapes degradation in the upper gastrointestinal tract. Probiotic genomes contain a large number of predicted carbohydrate modifying enzymes which reflects an apparent metabolic adaption to a complex carbohydrate rich environment.

In this study we aim at a molecular understanding of the selective glycan fermentation by different probiotics in the gut through glycoside hydrolase family 42 (GH42), which is a prerequisite to understand the metabolic interplay amongst these and to design/produce potent prebiotic and other bio-active oligosaccharides. Thus we have produced seven different GH42 enzymes from three different probiotic organisms (*Bifidobacterium animalis* subsp. *lactis* Bl-04, *Lactobacillus acidophilus* NCFM, and *Bifidobacterium longum* subsp. *infantis* ATCC 15697) and are currently analysing these with respect to their different sub-specificities and three dimensional structures.

What we know:

Protein expression. Protein purification. Protein Engineering. Glycoside Hydrolases. Enzyme Kinetics. Transglycosylation. Large data set manipulation and analysis.

What we need:

Substrates containing terminal non-reducing β-D-galactose.

Abstract no 3

How Carbon Nanostructures Affect Differentiation of Dopaminergic Neural Stem Cell

Andrea Casci Ceccacci, Letizia Amato, Claudia Caviglia, Anja Boisen, Stephan S. Keller, Arto Heiskanen, Jenny Emnéus

Department of Micro- and Nanotechnology, Technical University of Denmark

Research area and Objectives:

The project is focused on the assessment of the effects of carbon nanostructures used as substrates on differentiation of human neural stem cells (NSC). Different geometries in the nanoscale-range will be tested to know the best topography for NSC growth and differentiation. This could provide useful information for improving stem cell therapy and clinical trial in regenerative medicine.

What we know:

Dopaminergic neurons in the *substantia nigra*, a region of the brain, play essential role in the control of voluntary movement. Dysfunctional or dying dopaminergic neurons cause the subsequent lack of dopamine in patients affected by Parkinson's disease [1,2], which is manifested as impairment in motor function. NSCs transplantation into the brain of people affected by Parkinson's disease is one of the suggested therapeutic approaches for restoring dopamine production[3]. Previous studies have shown that grafted dopaminergic neurons can re-innervate the *striatum*, release dopamine and become functionally integrated in the host, resulting in clinical improvement[2,5]. In the development of NCSs able to differentiate to a dopaminergic lineage, the main concern is how large a fraction of a cell population can show dopaminergic properties.
Human Ventral Mesencephalic(hVM1) cells, extracted from mesencephalic tissue, are a clinically relevant cell lineage which can generate high fractions of dopaminergic cells (17.2%) after differentiation[4,6], and have been used during our study.
Nanostructures may influence the behaviour of stem cells[7]. Thereby, when using nanostructured surfaces as substrates for cell growth and differentiation, it is important to understand how stem cell fate can be directed in order to increase the number of dopaminergic cells.
In this study we combine a patterning technique for tuning the geometry of polymeric nanopillars (height, width, and spacing) with their further carbonization to obtain carbon nanopillar structures.

What we need:

The obtained carbon nanostructures are conductive and thereby may be used for evaluating dopamine exocytosis from dopaminergic cells in response to variable differentiation conditions, such as the substrate topography, as well as to follow their growth and differentiation. This could help in understanding how differentiation of neural stem cells has been affected by the kind of substrate they are growing and differentiating on. SEM imaging as well as confocal imaging will be further means to assess the cell morphology and the expression of relevant differentiation markers.

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Abstract no 4

New Insights Into the Substrate Specificities of Proton-coupled Oligopeptide Transporters from *E. coli* by a pH Sensitive Assay

Bala K. Prabhala[1], Nanda G. Aduri[1], Johanne M. Jensen[1], Heidi A. Ernst[1], Nida Iram[2], Moazur Rahman[2], Osman Mirza[1*]

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Abstract

Proton-coupled oligopeptide transporters (POTs) are secondary active transporters that facilitate di- and tripeptide uptake by coupling it to an inward directed proton electrochemical gradient. Here the substrate specificities of *E. coli* POTs YdgR, YhiP and YjdL were investigated by means of a label free transport assay using the hydrophilic pH sensitive dye pyranine and POT overexpressing *E. coli* cells. The results confirm and extend the functional knowledge on *E. coli* POTs. In contrast to previous assumptions, alanine and trialanine are substrates of YjdL, *albeit* poor compared to dipeptides. Similarly tetraalanine are a substrate of both YdgR and YhiP.

Abstract no 5

Tomato Thymidine Kinase Is Under Poor TTP Feed-Back Regulation

Birgitte Munch-Petersen[1], Nicolai Balle Larsen[2] and Jure Piskur[2].

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[2]Biology, Lund University, Lund, Sweden

Research area – enzymes for gene-therapy of Malignant gliomas

Malignant gliomas have a poor prognosis, and conventional treatment is of limited effect. Therefore, other therapeutical approaches such as gene-therapy with deoxynucleoside kinases are under investigation and the ongoing research is focused on identifying a dNK/pro-drug system, where the prodrug in the malignant cells is efficiently converted to the phosphorylated toxic compound. Recently, we reported a very promising candidate system for such a strategy: The thymidine kinase 1 from tomato (toTK1) combined with the nucleoside analog pro-drug zidovudine (azidothymidine, AZT), which is known to penetrate the blood-brain barrier.

What we know:

Transduction with toTK1 has been found to efficiently increase the sensitivity of human glioblastoma cells to AZT, and nude rats with intracranial glioblastoma grafts have shown significantly improved survival when treated with the toTK1/AZT system. This strong suicidal effect may partly be explained by the unique ability of toTK1 to phosphorylate AZT to the diphosphate level. However, other factors may play a role. Therefore, we investigated the effect of feed-back inhibition by TTP on AZT phosphorylation. For this purpose we prepared thymidine kinase 1 from tomato and human using the glutathione S-transferase (GST) expression system, purified and thrombin cleaved in situ on glutathione-sepharose. Enzyme activity was measured with a radio-assay using the DE-81 filter paper method. Feed-back inhibition with TTP was determined by measuring the activity at 10 μM tritium labeled thymidine or AZT as substrates and various concentrations of TTP. IC50 values (TTP concentration at 50% inhibition) were determined from Dixon plots.

We found that TTP is an efficient feed-back inhibitor of human TK1 (huTK1) with IC50 values of 2.9 μM and 6 μM at 10 μM thymidine and AZT, respectively. In contrast, toTK1 is poorly inhibited by TTP with manifold higher IC50 values: 38 μM and 42 μM at 10 μM thymidine and AZT, respectively.

What we need

We want to improve the toxic effect of drug-enzyme combination in gene-therapy with deoxynucleoside kinases. For this purpose we isolated a thymidine kinase type 1 from tomato with high capacity for AZT phosphorylation and found that this enzyme had a higher suicidal effect on transduced glioma cells compared to other thymidine kinases. Our investigations showed that this effect may be due to a lesser feed-back inhibition by the end-product of the thymidine kinase pathway – thereby producing more toxic AZT nucleotides.

Khan, Z., W. Knecht, M. Willer, E. Rozpedowska, P. Kristoffersen, A. R. Clausen, B. Munch-Petersen, P. M. Almqvist, Z. Gojkovic, J. Piskur and T. J. Ekstrom, Plant thymidine kinase 1: a novel efficient suicide gene for malignant glioma therapy, *Neuro. Oncol., 12*(6), 549-558, 2010.

Abstract no 6

DNAJB6 is a peptide-binding chaperone which can suppress amyloid fibrillation of polyglutamine peptides at substoichiometric molar ratios

Emanuelsson, Cecilia[1], Månsson, Cecilia[1], Melki, Ronald[2], Härmark, Johan[3], Kampinga, Harm H[4]

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Brief description of research area:

We investigate protein-protein interactions between molecular chaperones and client proteins in order to understand the molecular details of how they function. We focus on two chaperones, which both are oligomeric proteins: Hsp21 is a chloroplast small heat shock protein in *Arabidopsis thaliana* and one is a Human Hsp40-protein, DNAJB6.

What we know:

Protein expression and purification, cross-linking mass spectrometry, biochemical methods, spectroscopy, electrophoresis, immunoblotting.

What we need:

Arabidopsis genetics, human cell lines, setting up new methods like SPR and HDX.

Abstract no 7

Bioimpedance based Lab-on-a-Chip (LOC) systems for tissue engineering

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Brief description of research area:

The goal of our research is to develop a new proof of concept Lab-on-a-chip (LOC) system with integrated bioimpedance detection for the purpose of *real time monitoring* of the growth and viability of a vascularized 3D bioartificial liver-on-a-chip system (BAL-on-a-Chip). This project will be executed in synergy with the newly approved EU project NanoBio4Trans, which forms a new research direction within Nanotech's LOC strategic field.

The ultimate goal of the NanoBio4Trans project is to develop, optimise and validate a highly vascularised *in vivo*-like BAL as an extracorporeal bioartificial liver (EBAL) ready to be perfused with human blood plasma, in order to be exploited in the medical technology of the 21th century.

What we kow:

The use of *human induced pluripotent stem cells* (hiPSC) can be exploited as the starting material to enable the construction of personalized artificial organs from a patient's own cells. These cells can be grown and directed to differentiate into *in vivo*-like BALs by employing scalable and perfusable hybrid three dimensional scaffolds.Integrated optical and electrical biosensing systems can be used to monitor the effects and changes that occur during tissue growth. This allows control and surveillance of the BAL formation, with envisaged feed-back control.

Different bioimpedance-based 3D tissue culture Lab-On-A-Chip (LOC) systems are designed and optimized in order to follow cellular dynamics under *in vitro* growth conditions mimicking the *in vivo* environment. Since important electrical characteristic of an electrode/tissue system are determined solely by geometrical configuration, simplified finite element models can be used to optimize electrodes number and orientation and the deriving sensitivity field distribution inside the LOC. Different combinations of either current□carrying (CC) and pick□up (PU) electrodes or sensing configurations can be exploited. This approach can pave the way towards electrical impedance tomography (EIT) applications in order to image the changes in 3D cell culture environments.

Intra- and extracellular optical sensors will be also developed and applied for a multi-parametric imaging and bioanalysis of cells, tissues and organs (integrity of vasculature, viability, O_2, pH, liver function, differentiation markers, etc) working in close collaboration with *Luxcel Biosciences* (luxcel.com).
The perfusable hybrid scaffold and sensing systems will be integrated into the BAL support system enabling *real time monitoring* and *control* of the effects of various parameters during its growth.

What we need:

We need knowledge and techniques to:

1. establish and optimise highly sensitive *imaging* protocols for detecting hiPSC fate during their differentiation into hepatocytes and endothelial cells inside the scaffold.
2. develop optimised 3D human liver tissue culture systems, using i) innovative *3D cell culture formats*, and ii) *fluidic* BAL-on-a-Chip systems with specially adapted microenvironment conditions for hiPSC specific differentiation. The scaffold must consist of a primary highly dense and branched blood vessel-like channel network through which a heart-mimicking pulsating flow supplies nutrients and oxygen, surrounded by a secondary porous structure enclosing hydrogel micro deposits (HMDs) that contain necessary growth factors and signaling molecules. For those purposes it is crucial to improve the well established 2D design approach in order to realize a more sophisticated 3D microfluidic device, exploiting innovative and rapid technologies (e.g. 3D printing). Moreover the development of new biocompatible polymers that possess a combination of improved physico-mechanical properties is necessary.
3. adapt and *upscale* the optimised 3D growth and differentiation protocols to the BAL support system for growing BALs with dimensions in the order of cm^3 to dm^3.
4. *perfuse* the BAL with human blood plasma and test its ability for clearance of ammonia and bilirubin and production of certain key proteins.

Abstract no 8

Swine plasma immunoglobulins for prevention and treatment of post-weaning diarrhoea: Optimizing stability towards gut conditions

Chris Juul Hedegaard[1], Anne-Sofie Ballegaard[1], Nanna Røjel[1], Marie Bendix Hansen[2], Bodil Kjær Lindved[3] Kirsten Bisgaard Frantzen[4], Lars E. Larsen[1], Allan Lihme[2], and Peter M.H. Heegaard[1] –

[1]National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark. [2]Upfront Chromatography A/S, Copenhagen, Denmark. [3]KiBif ApS. [4]Multimerics ApS.

Brief description of research area:

A common problem in swine production is diarrhoea in newly weaned piglets, and huge quantities of antibiotics go to treat post-weaning diarrhoeas in piglets. The use of antibiotics can lead to the development of multi- and fully resistant bacteria, which consequently pose a great threat to human health. Therefore, sustainable alternatives for treating post-weaning diarrhoea without using antibiotics are in demand. Swine that are old (and big) enough for slaughter have during their upbringing been challenges by many different pathogens and thus have developed immunity towards these pathogens, which include pathogen-specific immunoglobulins (antibodies). We hypothesis that by harvesting natural immunoglobulins from porcine blood plasma, a waste product from swine slaughter, and feeding these immunoglobulins to the piglets this can subsequently (by passive immunisation) prevent and treat post-weaning diarrhoea.
Our challenge is to find a suitable method for stabilising the immunoglobulins for oral provision in order for the immunoglobulins to pass as unharmed as possible through the digestive system and still retaining their anti-pathogenic properties.

What we know:

It is possible to multimerise immunoglobulins, which results in an advantage when binding to their respective antigens in comparison to the non-multimerised immunoglobulins, but too high degree of multimerisation abates immunoglobulin reactivity. Unfortunately, a preliminary study showed that multimerisation destabilises the immunglobulins. On the other hand, proteolytical resistance correlates with increased immunoglobulin concentration.

What we need:

To investigate the effect of increasing the concentration of multimerised immunoglobulins on proteolytical resistance.
To investigate multimerised immunoglobulins' ability in inhibiting microbial (E. coli) adhesion on relevant matrices, such intestinal villi and/or intestinal cell lines.
A toxicological study on (if any) adverse side effects occurs when enteral providing immunoglobulins to piglets.

Abstract no 9

Structural and Enzymatic Characterization of Starch Phosphorylase from Barley

Christian Ruzanski, Katarzyna Krucewicz, Jose A. Cuesta. Seijo, Monica Palcic

The Carlsberg Laboratory, Gamle Carlsbergvej 10, KøbenhavnV

Brief description of research area:

Starch phosphorylases are important enzymes in the biosynthesis of starch in starch storing organs like potato tubers, rice, whea to initiate starch biosynthesis and only uses glucose-1-phosphate as donor substrate to produce t and barley endosperm. Results indicate the enzyme might not need an acceptor substrate and elongate glucans.

What we know:

- We have the structure of the enzyme
- We know its redox controlled
- We know how to control dimerisation of the enzyme

What we need:

- How can we know that no glucan impurity in the preparation causes “de nove” synthesis of glucans?
- The structure is incomplete, a flexible loop is missing, how can we analyse the structure of the flexible loop?
- The enzyme is dimeric (in the crystal) and seemingly trimeric in solution. Deleting the flexible loop changes the oligomeric state of the enzyme to monomeric – the loop however is not part if the dimer interface – Why?

Abstract no 10

Phosphate and Arsenate Binding in P-Loops

Elizabeth Wood[1], Mathias F. Gruber[1], Andrea Bordoni[2], Claus Helix-Nielsen[1]

[1]Physics, Technical University of Denmark. [2]Institute for Mathematics and Computer Science, Technical University of Denmark.

Brief description of research area:

Multi-scale computational modeling of phosphate/arsenate binding by proteins (Walker A Motifs) and small (synthetic) peptides.

What we know:

We have conducted molecular dynamics simulations of binding. We have computational models of the conformations of our small synthetic peptide. We have expanded the AMBER force field to allow for computational models that include binding with arsenate, in addition to phosphate.

What we need:

We need experimental confirmation of our findings, including a way of approaching the characterization of very small, highly flexible synthetic peptides in their bound and unbound state. We need binding assays that characterize the binding affinity these molecules have towards phosphate and arsenate.

Abstract no 11

LC-MS based differential proteomics as tool for high throughput assessing differences in plant and microbial research

Giuseppe Dionisio[1]

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Brief description of research area:

I am working with LC-MS based proteomics in cereal like barley, wheat and rye. I study hydrolytic enzyme activation by reductants and proteolytic activation. The workflow I adopt is to start from studying the proteome of a particular set of enzymes (i.e. proteases) and verify *in vitro* their peptide mapping of the inactive (dormant grain) versus the active enzyme (germinating grain). Such differences will be used for expressing as recombinant the enzyme in question in *Pichia pastoris* in order to control its active status. I am working with purple acid phosphatases, plant proteases, plant and fungal xylanases and plant xylanase inhibitors. Furthermore my recent interests embrace the cereal reserve proteins and also metal storage proteins in grains.

What we know:

Gel based proteomics can effectively help in the case of differences occurring at macroscopic level and only with the use of robot is high throughput. Anyhow this above technique has been today improved by the use of liquid chromatography coupled to mass spectroscopy. This last "Omics" LC-MS based differential proteomics can effectively reduce the hand in workflow and speed up the comparison of many samples or conditions.

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 Tribrid 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 *in silico* many spectra. Besides, new algorithms based on GPU computing are more and more used to speed up the bioinformatics of the MS calculus. Finally the increase of label free differential proteomics is lately predominating since the quantification targeted (quantitative proteomics) is also performed by PRM (parallel reaction monitoring) feature of the Orbitrap MS instruments. Since Odense has represented so far a center for MS with emphasis on human proteomics it is now time that a MS modern center will be created for plant or microbial research.

Abstract no 12

Residual Structure in Unfolded Proteins

Heike I. Rösner[1], Birthe B. Kragelund[1].

[1]University of Copenhagen

Brief description of research area:
Protein folding and misfolding

What we know:
There is residual structure in the unfolded state of proteins. This residual structure is often non-native like. Even though proteins might share a common fold, the residual structure shows no apparent motif conservation.

What we need:
We would like to find ways to characterise the residual structure in addition to NMR spectroscopy. We would like to find out if the residual structure is still somehow conserved but maybe not in the classical terms. The ultimate aim is to find out what role this residual structure is playing in folding and misfolding.

Abstract no 13

Structural, functional and kinetic characterization of Lymphostatin, a potential glycosyltransferase toxin from *E. coli*

Husam Alsarraf[1], René Jørgensen[1]

[1]Statens Serum Institute

Pathogenic bacterial strains, such as *Clostridium difficile, Vibrio cholera*, enteropahogenic and enterohemorrhagic *E.coli* (EPEC and EHEC, respectively), often rely on virulence factors to facilitate infection and colonization in their host targets. The recently identified virulence factor, lymphocyte inhibitory factor A (LifA), encodes for the large toxin called lymphostatin. Lymphostatin is a 360 kDa protein containing three putative enzymatic motifs; a glycosyltransferase, a protease and an aminotransferase motif. It is found in enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *E. coli* strains, in *Chlamydia sp* and *Citrobacter sp* and is coupled with the ability to colonize the intestines of animals.

In addition, lymphostatin is capable of altering the proliferation of lymphocytes and the production of certain immunologic signal molecules upon infection. Yet the exact function of this protein is unknown. However, based on structural predictions, it is hypothesized that lymphostatin is a glycosyltransferase, that by adding a sugar molecule to host cell GTPases, alters the host cell functions.

Studying the structure of LifA, particularly the glycosyltransferase domain, by means like protein crystallography, small angle x-ray scattering and other methods combined with kinetic studies may give us insight into how LifA alters the host GTPases in order to enhance the survival of the infecting pathogenic *E.coli*.

Abstract no 14

HEXPIN: Hetero-exopolysaccharide–milk protein interactions

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Brief description of research area:

Chemically modified polysaccharides from plants or seaweeds 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 strains have a similar 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, which makes it highly attractive in the developing trend towards healthier food products with fewer additives.

The textural property of fermented milk products is an effect caused by complexation between HePS and milk proteins. However, the correlation at the molecular level between HePS-milk proteins interactions and the textural properties are poorly understood. The project aims at characterizing the interaction between known structurally diverse HePS and various milk proteins using biophysical, micro- and macroscopic analyses. HEPS oligosaccharide 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 select by choice specific HePS to achieve desirable textural properties, e.g. sensory creaminess, ropiness and better structural rebuilding of fermented milk products , without the use of additives.

What we know:

- Homo-exopolysaccharides (HoPS) composed of various linear and branched α-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.
- Surface plasmon resonance (SPR) is suitable to analyze binding constants and rates of oligosaccharide-protein interactions and has successfully characterized milk protein binding to HoPS of different known structures (Diemer *et al.* 2012).
- We have established a database describing the structural composition and textural properties of known HePS repeat units.

What we need:

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

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

Reference:
Diemer SK, Svensson B, Nygren Babol L, Cockburn D, Grijpstra P, Dijkhuizen L, Folkenberg DM, Garrigues C, Ipsen R (2012) Binding interactions between -glucans from Lactobacillus reuteri and milk proteins characterised by surface plasmon resonance. Food Biophys 7, 220-6

Abstract no 15

Structure of CLG1_GBSS, a starch synthase from a cyanobacterium

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Brief description of research area:

Starch synthesis used to be thought exclusive of archaeplastida, which include green algae and land plants. Recently, amylopectin-like polymers have been identified in group V cyanobateria[1]. In particular, a newly isolated cyanobacterium, CLG1, synthetizes granules containing both amylose and amylopectin essentially identical to plant starch. These cyanobacteria are proposed to have contributed key enzymes that enable plants to synthetize starch rather than glycogen.

Here we report the crystal structure of GBSS, the granule bound starch synthase responsible for amylose synthesis in CLG1, in complex with ADP and acarbose or glucose. The structure reveals different conformational states of the ternary complex and is compared to plant starch synthases and to glycogen synthases.

What we know:

Recombinant expression, purification, characterization and crystallization of enzymes in the starch pathway.

What we need:

Modeling, both at the protein and polymer levels, to understand the link between modifications in enzymatic activity and modifications in the properties of the resultant starch.

Abstract no 16

Recombinant Production of Human Aquaporin-1 to an Exceptional High Membrane Density in *Saccharomyces cerevisiae*

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Brief description of research area:

The yeast *Saccharomyces cerevisiae* was explored for the capacity as host for heterologous expression of human Aquaporin-1. Aquaporin-1 cDNA was expressed from a galactose inducible promoter situated on a plasmid with an adjustable copy number. Human Aquaporin-1 was C-terminally tagged with yeast enhanced GFP for quantification of functional expression, determination of sub-cellular localization, estimation of *in vivo* folding efficiency and establishment of a purification protocol. Aquaporin-1 was found to constitute 8.5 percent of total membrane protein content after expression at 15°C in a yeast host over-producing the Gal4p transcriptional activator and growth in amino acid supplemented minimal medium. In-gel fluorescence combined with western blotting showed that low accumulation of correctly folded recombinant Aquaporin-1 at 30°C was due to *in vivo* mal-folding. Reduction of the expression temperature to 15°C almost completely prevented Aquaporin-1 mal-folding. Bioimaging of live yeast cells revealed that recombinant Aquaporin-1 accumulated in the yeast plasma membrane. A detergent screen for solubilization revealed that CYMAL-5 was superior in solubilizing recombinant Aquaporin-1 and generated a monodisperse protein preparation. A single Ni-affinity chromatography step was used to obtain almost pure Aquaporin-1. Recombinant Aquaporin-1 produced in *S. cerevisiae* was not N-glycosylated in contrast to the protein found in human erythrocytes.

What we know:

Recombinant production of human aquaporin-1 to exceptional high membrane density in yeast

What we need:

Large scale purification expertise, fermenter expertise

Abstract no 17

Detailed Time Resolved Immunologic Characterization of Starch Active Enzymes throughout Barley Endosperm Development

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Brief description of research area:

Crop plants like barley invest a large proportion of their photosynthates into starch biosynthesis. Starch synthases (SS), starch branching enzymes (SBE) as well as starch debranching enzymes (SDBE) are the main drivers of starch biosynthesis in plants. The timely interplay of all these enzymes ensures efficient synthesis of starch throughout barley endosperm development. So far, only transcript analysis was done to analyze the quantity of each enzyme at any given point during development. Since transcripts do not necessarily mirror protein production we undertook a complete immunologic analysis of all starch synthases and branching enzymes during barley endosperm development.

What we know:

All SSs are produced at the same time with a sudden onset of production

- SBEIIa seems to be produced right from the start of endosperm development

What we need:

- All Immunoblots where optimized efficiently, however – Anti-SSIIIa and AntiSSIIIb peptide antibodies have not performed well at all, any clues on how to improve this?
- Zymograms have not been working on most of the SSs, no activity or very little. Any clues on enzyme stability in activity gels?
- All soluble starch synthases localized to the buffer insoluble protein pellet – is that the true nature of barley soluble starch synthases or rather protein extraction issues?

Abstract no 18

Redox regulation of starch biosynthetic enzymes in *Arabidopsis thaliana*

Katsiaryna Skryhan[1], Mikkel A. Glaring[1], Brian B. Nielsen[1], Morten M. Nielsen[2], Jose A. Cuesta-Seijo[2], Monica M. Palcic[2], Andreas Blennow[1]

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Brief description of research area:

We performed a comprehensive analysis of the redox sensitivity of known starch biosynthetic enzymes in leaf extracts of *Arabidopsis thaliana* using native activity gels and enzyme assays. Our results confirmed that at least three starch biosynthetic enzymes are activated by reduction at physiologically relevant potentials - starch synthases SS1 and SS3, and starch branching enzyme BE2. We chose these three enzymes to be expressed as 6xHis-tagged proteins in *E. coli* cells. An analysis of purified recombinant proteins confirmed redox sensitivity of SS1 and SS3 but not BE2.

To identify cysteines that are involved in the formation of the regulatory disulfide linkage of 6His-SS1 protein, we generated cys-to-ser site-specific mutants, one for each cysteine present in the sequence.

Enzyme assay data reveal that cys 164, cys 217 and cys 545 are involved in redox sensitivity and activity of SS1.

What we know:

Native activity gels and enzyme assays for monitoring activity of starch synthases and branching enzymes (both recombinant proteins and from plant extract)

What we need:

Test affinity of SSs and BE to Trxs and NTRC

Abstract no 19

Binding of the N-terminal domain of the lactococcal bacteriophage TP901-1 CI repressor to its target DNA: a crystallography, small angle scattering and NMR study

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*Corresponding author, # Both authors contributed equally to this work

Brief description of research area:

Phage TP901-1 is a temperate bacteriophage that has the option of either of two lifecycles, namely the *Lysogenic* lifecycle or the *Lytic* Lifecycle. In the *Lysogenic* lifecycle the bacteriophage incorporates its viral DNA into the host genome and remains dormant, until adequate stimuli switch transcription towards the *Lytic* Lifecycle. The choice is determined by the *bistable genetic switch*, which is controlled by a hexameric form of the CI repressor protein binding to operator sites on the DNA. The CI consists of two functional domains, namely an N-terminal domain (NTD) responsible for DNA binding through a helix-turn-helix (HTH) motif, and a C-terminal domain (CTD) involved in oligomerization of the monomers.

What we know:

We cloned, expressed and purified CI proteins (native, truncated versions and mutants hereof). Crystallization trials, both screens and optimization, of proteins as well as protein-DNA complexes were performed. Recently the X-ray crystal structures of NTD and an NTD-DNA complex were solved. The structures revealed an extension of one of the helices in the HTH motif, not previously observed in related phages. The extension is speculated to be involved in protein-protein interactions and thus to hold a functional role in the control of the genetic switch.

What we need:

Methods for studying protein-DNA and/or protein-protein interactions, to potentially elucidate the function of the extended helix and its role regarding the bistable genetic switch.

Abstract no 20

Exploring the Secretomes of Starch and Chitin Degrading Fungi

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Brief description of research area:

Chitin and starch are two of the most abundant polysaccharides in nature next to cellulose and are widely used in industrial applications including biofuels, pharmaceuticals, textiles, paper, and food[1-2]. In contrast to lignocellulose matrices[3], much less effort has been imparted on discerning the enzyme cocktails deployed by fungi, both with respect to class, composition and proportion, for the degradation of chitin and starch. This insight is likely to have considerable impact on the efficiency of industrial processing of these polysaccharides. Fungi are renowned for producing an arsenal of carbohydrate activity enzymes[4] (CAZymes) to harvest energy from various glycans and to potentiate infection (in case of plant pathogens).

The main focus of the present study is to examine the degradation machineries of chitin and starch by various fungal taxa based on their ecology. The project will involve *in silico* analyses of the fungal CAZymes plausibly involved in the degradation of chitin and starch. This will be a basis to examine the growth of selected strains on these substrates and use proteomics analyses and activity measurements to probe the expression levels of the enzyme consortium targeting starch and chitin.

Literature

1. Eijsink V.G., *et al.*: *Trends Biotechnol*, (2008) 26:228–235.
2. Alvani K., *et al.*: *Starch-Stärke*, (2012) 64:297–303.
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What we know:

Secretomics. Protein expression. Protein purification. Protein Engineering. Protein characterization.

What we need:

Differential transcriptomics of fungi grown on complex polysaccharide substrates. Mapping evolutionary relationships of relevant enzymes classes in fungi to study evolution/co-evolution of proteins deployed for the same function.

Abstract no 21

SRM and QconCAT based analysis of cytokines, acute phase proteins and hypoxia related proteins in the horse

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Brief description of research area:

We are working with animal proteomics. The project presented here is within the field of equine proteomics. The work has emphasized the establishment of an equine PeptideAtlas and the development and validation of methods for absolute quantification of inflammatory and hypoxic proteins in horse tissues and body fluids, using SRM based methods and quantification concatamer (QconCAT) based production of standard peptides.

What we know:

Knowledge about design and validation of SRM methods.
Knowledge about how to handle quadrupole TOF, triple quadrupole instruments, and nanoLC systems.
We have two QconCATs – one targeting equine acute phase proteins, one targeting equine cytokines, growth factors and hypoxia-related proteins.
Knowledge about clinical experimental studies and the veterinary field (educated as veterinarian).

What we need:

Post Doc position / collaborators for future projects within the field of human or veterinary clinical proteomics.

Abstract no 22

Characterizing mikro- and nanostructures in soft and wet samples

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Brief description of research area:

Micro- and nanostructural characterization plays an increasing role in all areas of science. Additionally there is an increasing demand for compositional analysis or identifying specific particles within samples. Electron microscopy is versatile and can be utilized for many of these aspects. For samples that contain water or can be describes as soft; such as biological samples, environmental samples and food samples, the preparation for electron microscopy can be quite challenging and artifacts from the preparation can be misinterpreted for real data. At DTU Cen (DTU Center for Electron Nanoscopy) we are working with several of these preparation and microscopy methods, such as cryo microscopy and environmental SEM. We are now also setting up lab facilities for chemical preparations and hybrid techniques such as freeze substitution.

What we know:

DTU Cen was founded almost six years ago by a 100 million DKK grant from the Maersk foundation. We are a core facility for electron microscopy in DTU and have collaborations with a wide selection of institutes in DTU as well as internationally. We pt have seven electron microscopes: SEMs, TEMs and dual beam microscopes, which in combination enable us to bridge the gap from low resolution down to atomic resolution. At Cen we are working with several of the preparation and microscopy methods for soft and liquid samples, such as cryo microscopy and environmental SEM. We are now also setting up facilities for chemical preparations and hybrid techniques such as freeze substitution.

What we need:

We are interested in scientific collaborations in characterization of soft / liquid samples and biomimetic materials as well as in networking.

Abstract no 23

The membrane proximal region of the intracellular part of the human growth hormone receptor is intrinsically disordered

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The growth hormone receptor (GHR) plays a role in the regulation of growth at a cellular and a systemic level. A dysregulation of the GHR can lead to acromegaly, a disease also known as gigantism, or the syndrome Laron-type dwarfism. Apart from regulating growth, the GHR is an important metabolic modulator and is therefore suggested to play a role in diabetes [1]. Furthermore, a link between the GHR and cancer is suggested [2]. Thus, the GHR plays a role in multiple diseases. To fully understand the function of the GHR in health and disease a mapping of its structure, interactions and dynamics is indispensable towards an understanding of the hormone-induced signal transduction.
The GHR is a single-pass transmembrane protein belonging to the type I cytokine receptor family. It dimerizes in the membrane prior to binding of the ligand and the extracellular domains are known to interact with growth hormone (GH). This interaction involves two receptor molecules and one hormone. Binding of GH is shown to cause conformational changes in the transmembrane domain which is thought to initiate signal transduction through the intracellular domain (ICD) [3]. The ICD interacts with JAK2 and this mediates signaling downstream from the GHR [4]. The conformational changes in the transmembrane domain that occur upon binding of GH to the receptor activate JAK2 and initiate activation of the JAK-STAT pathway. The structure of the ICD of the GHR (GHR-ICD) is not yet known.

What we have done:

We have investigated the possibility that the ICD of the human GHR is disordered. Using bioinformatics tools as well as biophysical techniques, including circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy, we have shown that the membrane proximal part of GHR-ICD (GHR-ICDmp) is partly disordered, but with regions of transient secondary structure. These are generally the same regions predicted to interact with binding partners. Furthermore, we have investigated the interaction of GHR-ICDmp with hallmark lipids of the inner leaflet of the plasma membrane, also using CD and NMR spectroscopy. These data suggest that GHR-ICDmp interacts with the plasma membrane.

What we would like to do:

We would like to know how the GHR decides which signal transduction pathway to activate and how the regulation of this activation is mediated. As we do not know all downstream targets of GHR activation it is hard to verify the biological function of a certain interaction or posttranslational modification.

Abstract no 24

One-pot enzymatic production of GalNAc from GlcNAc

Mamoru Nishimoto[1, 2], Kousuke Inoue[1], Motomitsu Kitaoka[1].

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[2] Carlsberg Laboratory

Brief description of research area:

Enzymatic synthesis of oligosaccharides has been developed by hydrolysis of cheap substrates such as starch and sucrose. Because oligosaccharides that can be prepared in this method are limited by the starting materials, it is often impossible to produce a desired oligosaccharide. On the other hand, it is possible to prepare many kinds of oligosaccharide selectively using sugar nucleotide glycosyltransferases or sugar phosphorylases. However, the disadvantage of these two enzyme classes for oligosaccharide synthesis is the cost of substrate. We therefore aim to construct an enzymatic reaction that combines multiple enzymes and cheap starting materials for the synthesis of desired oligosaccharides. In this poster presentation, we focused on the reaction of UDP-Glc: HexNAc 1-phosphate uridylyltransferase [EC 2.7.7.12] and constructed a conversion reaction from inexpensive GlcNAc to expensive GalNAc using three enzymes in a one-pot reaction.

What we know:

I have prepared 1.4 kg of Lacto-*N*-biose I (LNB, Gal β1,3 GlcNAc) and 45 g of Galacto-*N*-biose (GNB, Gal β1,3 GalNAc) from sucrose and GlcNAc/ GalNAc using four enzyme reactions containing sugar phosphorylase in a one-pot reaction. Both LNB and GNB are growth factors for bifidobacteria, whereas LNB is the major carbohydrate present in human milk oligosaccharides and GNB is the solely core 1 sugar in mucin. We are able to provide LNB and GNB for your research in gram scale.

What we need:

I study oligosaccharide synthesis using a sugar nucleotide glycosyltransferase at the Carlsberg Laboratory (Prof. M. Palcic group) as a visiting researcher. I am seeking a way to produce sugar nucleotides at low cost.

Abstract no 25

Features of oligopeptidase B from *Serratia proteamaculans*

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OpdB is a trypsin-like serine proteinase found in ancient unicellular eucaryotes, such as trypanosomes *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Trypanosoma evansi* and leishmaniae *Leishmania major* and *Leishmania amazonensis*. Genes encoding this enzyme are also found in gram-negative pathogenic bacteria such as *Escherichia coli*, *Moraxella lacunata*, *Salmonella enterica*, mycobacteria *Mycobacterium tuberculosis* and *Mycobacterium leprae*, and spirochete *Treponema denticola*. Oligopeptidases B are important virulence factors in trypanosomal infections such as Chagas disease and African sleeping sickness. Parasitic OpdB catalyze abnormal degradation of host peptide hormones such as atrial natriuretic factor, thus being implicated in pathogenesis of different trypanosomiases and leischmaniases. It should be emphasized that genes encoding this enzyme are not found in mammals. Thus, OpdB of protozoan parasites may serve as therapeutic targets in search for pharmaceuticals against these dangerous infections. Prokaryotic homologs of this protein are far less studied, but are also supposed to be important targets for antimicrobial chemotherapy.

Analysis of the regulation of the thermoinactivation of new psychrophilic enzyme – oligopeptidase B from S*erratia proteamaculans* (PSP) was carried out. The influence of temperature and composition of the reaction medium from *Escherichia coli* on the rate of the enzyme inactivation was studied. The comparison of inactivation rates of PSP and mesophilic enzyme trypsin at 37-50°C was carried out. The psychrophilic character of PSP was demonstrated: this enzyme lost its activity rapidly in 0,1 M Tris-HCl-buffer pH 8,0 already at 37°C in contrast to comparatively stable trypsin. The effect of PSP thermostabilisation by glycerin at 37-50°C was revealed. Calcium ions and buffer solution of low molarity cause the opposite effect – the acceleration of PSP inactivation at increased temperatures.

The *S. proteamaculans* 94 *OpdB* gene was sequenced and the producer strain *Escherichia coli* BL-21(DE3) pOpdB was constructed. The amino acid sequence of oligopeptidase B from *S. proteamaculans* (PSP) closely resembles those of oligopeptidases B from *E. coli* and *S. enterica*. An interesting feature of the primary structure of *S. proteamaculans* oligopeptidase B is the absence of one residue of the Asp/Glu pair controlling P2-specificity of the enzyme: Asp462 is substituted by Ala. In trypanosomal and some bacterial oligopeptidases B, this pair Asp/Glu 460 and 462 is obligate. The most homologous to the PSP amino acid sequence is that of OpdB (deduced from the gene sequence) from *Yersinia pestis* biovar *Microtus str*. 91001 (causative agent of plague), in which the negatively charged residue 462 is also absent.

Abstract no 26

Proteins propensity for cholesterol-enriched biomembrane domains, the so-called‘rafts’. Bioinformatics predictions

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Research area:

The physical and physico-chemical properties of bio-mimetic membranes. Investigation by modeling and computer simulations, and a bioinformatics method.

What we know:

The term 'raft' is used to denote small (10-200nm), heterogeneous and highly dynamic biomembrane domains enriched in cholesterol and sphingolipids where proteins are embedded or attached (Pike, 2006; Lingwood and Simons, 2010; Rheinstädter and Mouritsen,
2013). The affinity of proteins for rafts may depend on features such as lipidation or GPI-anchoring (Pike, 2004). Can this affinity be inferred solely by knowing some protein 'features', thus neglecting the lipid environment?

What we need:

A bioinformatics method based on artificial neural network (ANN) training was used to recognize feature-based patterns in proteins (Jensen et al., 2002) that are known to be raft-proteins. The trained ANN was then utilised to make predictions about the raft-affinity of proteins, for which there is no a priori knowledge of their raft involvement. The aim of this presentation is to give an overview of the ANN feature-approach and to show some preliminary results on protein raft-affinity.

Abstract no 27

Insights gained from the crystal structure of the complex of barley limit dextrinase and barley limit dextrinase inhibitor

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Research area:

Plant seeds commonly constitute rich sources of numerous proteinaceous inhibitors of various polysaccharide degrading enzymes and proteases. Such inhibitors can have central regulatory roles or act in defence against pathogens.[1,2] Recombinant barley limit dextrinase (LD) of the α-amylase family (GH13) hydrolysing α-1,6 branch points from starch and its endogenous cereal-type inhibitor (LDI) interact very tightly with K_D of 40 pM due to an unusually slow k_{off} of $6 \cdot 10^{-5}$ s^{-1} as measured by surface plasmon resonance (SPR). Since LD is the sole debranching enzyme in the germinating seed, it gets a key role in malting and brewing. Inhibition by LDI, however, suppresses the degradation of branched limit dextrins to fermentable sugars.[3] We solved the crystal structure of the LD:LDI complex to 2.7 Å resolution. Despite the high structural similarity between LDI and three other structurally characterised cereal-type inhibitors acting on α-amylases and proteases, LDI only inhibits LD, as far as we can tell, and in doing so uses a binding mechanism completely different from the double-headed cereal-type inhibitors, e.g. as seen for the bifunctional α-amylase/trypsin inhibitor from ragi (RBI) acting on yellow meal worm α-amylase,[4] although α-amylase and LD are in the same glycoside hydrolase family. The loop of LDI involved in LD inhibition corresponds to the RBI's trypsin binding site, but the residues corresponding to the trypsin binding residues are not conserved. Based on the observations about the small differences at structure level, which makes a huge difference for inhibition specificity, LDI is suggested to be a backbone for computational protein design of inhibitors against other GH13 enzymes and proteases.

What we know:

Recombinant protein production in *Pichia pastoris* by high cell density fermentation.
Protein structure determination by X-ray crystallography.
Analysis of protein-protein interaction by surface plasmon resonance (SPR).

This work was supported by The Carlsberg Foundation, The Danish Council for Independent Research | Natural Sciences, DTU PhD stipends (to MVBC and MSM) and an Oticon foundation MSc scholarship (to JMJ).

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Abstract no 28

Multi-scale computational modeling of phosphate/arsenate binding by proteins (Walker A Motifs) and small (synthetic) peptides

Elizabeth Wood[1], Mathias F. Gruber[1], Andrea Bordoni[2], Claus Helix-Nielsen[1]

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Brief description of research area:

Multi-scale computational modeling of phosphate/arsenate binding by proteins (Walker A Motifs) and small (synthetic) peptides.

What we know:

We have conducted molecular dynamics simulations of binding. We have computational models of the conformations of our small synthetic peptide. We have expanded the AMBER force field to allow for computational models that include binding with arsenate, in addition to phosphate.

What we need:

We need experimental confirmation of our findings, including a way of approaching the characterization of very small, highly flexible synthetic peptides in their bound and unbound state. We need binding assays that characterize the binding affinity these molecules have towards phosphate and arsenate.

Abstract no 29

Computational investigations and novel approaches in the study of the dynamics and structural behavior of Zn^{2+} binding proteins and intrinsically disordered proteins

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Brief description of research area:

Our current research area focuses on the study of propagation of structural signals in the dynamics of proteins and the relationship between structure, dynamics and biological function. We are employing a multidisciplinary approach, performing both computational techniques (as for example molecular dynamics (MD) simulations) and biophysical experiments. In particular we are applying these methods to target proteins of high relevance, due to their implications in human diseases.

What we know:

We have a large expertise in biomolecular simulations of different cases of study. We are, in particular, focusing on two main biological topics of research. A main project is related to the investigation of the dynamics properties of intrinsically disordered domains (IDPs) with particular attention to the role of their structural disorder in modulating their biological function. Another main area of research is the study of the structural effects induced by metal ions on protein structure and function, describing also the structural effects induced by non-essential metals. Our investigations strongly support the potential of MD-based approaches, compared with experimental data from biophysical experiments, such as Electro-Spray Ionization Mass Spectrometry (ESI-MS).

Other topics of interest are the regulation by phosphorylation of proteins involved in the ubiquitination pathway, as well as the study of cold-adaptation in enzymes isolated from psychrophilic organisms.

The integration of these methods provides an effective and accurate atomic-level characterization of the conformational landscape accessible to biomolecules. Moreover our results show that atomistic explicit-solvent MD simulation can successfully describe even complex phenomena, like IDPs and metal-binding proteins.

What we need:

Since our main projects rely on a multidisciplinary approach, employing molecular simulations combined to biophysical techniques, to develop further our research we are always looking for collaborations with other institutes and research groups in experimental structural biology. An experimental investigation, such as by NMR spectroscopy, would be of great utility and perfectly complementary to our work. Moreover our computational results give very useful insights that can be used to design experiments as the identification of residues for mutagenesis or insight on mechanisms that can be probed experimentally. It is known that some approximations are still present in the MD force-fields, as for example in the description of non-essential metals or the overcompaction of IDPs, and experimental data are essential in order to compare and validate our results generated *in silico.* We are also looking for collaborators with expertise in protein biochemistry to test *in vitro* and *in vivo* the mutants designed *in silico*.

We aim to develop further our multidisciplinary approach, increasing the capability to compare and integrate structural data collected from different biophysical techniques to the computational data. This would help in achieving a more comprehensive scenario of the system under investigation, which is essential for the effective description of protein structures, dynamics and their relationship with biological functions.

Abstract no 30

The Equine PeptideAtlas

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Brief description of research area:

We are working with animal proteomics. The project presented here is within the field of equine proteomics. The work has emphasized the establishment of an equine PeptideAtlas and the development and validation of methods for absolute quantification of inflammatory and hypoxic proteins in horse tissues and body fluids, using SRM based methods and quantification concatamer (QconCAT) based production of standard peptides.

What we know:

Knowledge about design and validation of SRM methods.
Knowledge about how to handle quadrupole TOF, triple quadrupole instruments, and nanoLC systems.
We have two QconCATs – one targeting equine acute phase proteins, one targeting equine cytokines, growth factors and hypoxia-related proteins.
Knowledge about clinical experimental studies and the veterinary field (educated as veterinarian).

What we need:

Post Doc position / collaborators for future projects within the field of human or veterinary clinical proteomics.

Abstract no 31

Exploring the specificity of two a-glucan associated ABC transport systems of *Bifidobacterium animalis subsp. lactis* Bl-04

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Brief description of research area:

Glycan utilization of the gastrointestinal tract is important in shaping the complex gut microbial community[1]. The balance of the microbiota is influenced by the host diet and affects host health and disease[2]. *Bifidobacterium* is an important genus with several strains identified as probiotics[3], beneficial to human health, and efforts are made to better understand their uptake and role in utilization of glycans in the gut. A recent transcriptomics study of oligosaccharide utilization in *Bifidobacterium animalis subsp. lactis* Bl-04 identified a cluster of genes containing two ATP binding cassette (ABC) transporters and α-glucan utilization enzymes[4]. This study aims to explore the specificity of these two ABC transporters by characterizing their specificity determining solute-binding protein (SBP). One SBP binds oligomers of α-1,4-glucopyranosides with maltotetraose as the preferred ligand at a K_D of 100 nM, consistent with the proposed role in uptake of α-glucan. The other SBP has been tested against a range of ligands, including all α-linked disaccharides of glucose, but is only showing significant binding to α-1,4 linkages, preferring maltose at a K_D of 600 μM. Further investigations, on determining the biologically relevant ligands of this ABC transporter, are under way.

What we know:

Cloning, production and purification of recombinant proteins.
Analysis of protein-carbohydrate interactions by surface plasmon resonance (SPR).
Analysis of affinities and thermodynamic parameters of binding by isothermal titration calorimetry (ITC)

What we need:

To analyse evolutionary relationships and conservation of genes between gut bacteria.

Acknowledgement:

This research was funded by a FøSu grant from the Danish Strategic Research Council to the project 'Gene discovery and molecular interactions in pre/probiotics systems. Focus on carbohydrate prebiotics'.

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Abstract no 32

Modulating β-glucosidase Zm-p60.1 specificity for natural substrates

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Plant biotechnology is critical for addressing the challenge of food security in the near future. In addition it has substantial potential in increasing energy supply, bioremediation as well as the production of important chemicals including, but not limited to, biomedicals.
Virtually all aspects of plant growth and development including key factors enabling the development of usable plant biotechnology are controlled by the plant hormone system.
Cytokinins are essential hormones for almost all stages of plant development and the most abundant natural cytokinin is zeatin. O-glucosylation is a method for temporarily inactivating zeatin and the resulting conjugate is stored in the vacuole.
The maize b-glucosidase Zm-p60.1 is important for plant development due to its role in the targeted release of free hormones (cytokinins) from their inactive storage forms - cytokinin-O-glucosides, (Brzobohatý et al, 1993; Kiran et al, 2006).
The aim of our research is to modulate enzyme specificity and to understand the functional relationships between key amino acid residues that form the entrance to the active site.

What we know:

Enzyme expression, purification and characterization.
Mutagenesis and screening (assay development)
Expression in plant system

What we need:

Gaucher disease research collaboration
Cryo-electron microscopy
High throughput screening and lab automation
FRET-fusion construction and analysis
Isolation of enzyme from plant material

Abstract no 33

Combining Small Angle X-ray Scattering with the Rosetta modelling suite for identification of the native-like structure of protein complexes

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Research area:

Protein interaction and stability examined by Small angle X-ray scattering and *in-silico* modelling.

What we know:

Small angle X-ray scattering (SAXS) can be used to identify the overall shape of proteins and protein complexes with known or partly known components, and *ab initio* models can be derived from the scattering profile using programs such as Dammif [1] and Gasbor [2] from the Atsas software package. Protein complexes, where the structure of only part of the components is known can also be investigated with programs such as Bunch [3], which combines rigid body modelling with *ab initio* shape reconstruction.

These tools give us knowledge of the overall conformation of the macromolecular structures, but cannot provide detailed information on the molecular level regarding protein-protein interfaces of the complexes. The Rosetta modelling suite is a multipurpose software suite originally developed for *de novo* structure prediction but today contains a variety of programs including the protein docking tool.

An inherent problem with the Rosetta docking tool [4] is the difficulties in identifying the native-like structure which should be among the lowest energy scoring complexes identified. In order to better identify the native-like structure, one can apply different constrains derived e.g. from biochemical knowledge, NMR [5] (nuclear overhauser effects or residual dipolar couplings) or SAXS (shape restrains).

What we need:

Currently, no benchmarking has been published regarding the relative success of using SAXS constrains though [6]. Sgourakis and co-workers [6] applied SAXS constrains in determination of symmetric oligomers. The authors concluded that although SAXS constrains narrow the pool of potential complex structures, the symmetry of the oligomers does impose a problem as it does in conventional SAXS docking programs. Oddly, the Rosetta energy term was not capable of differentiating between the symmetry inverted complexes.

We want to use SAXS constrains for determination of complexes of non-identical components using the Rosetta docking protocol. At first, a small pool of complex structures has been chosen to evaluate the difference between conventional docking (dock_conv) and docking performed using SAXS constrains (dock_SAXS). We do not intend to make a full benchmarking, but knowledge of the difference (dock_conv *vs*. dock_SAXS) and limitation of dock_SAXS is needed, before applying dock_SAXS to unknown complex structures. Examples will be presented in the poster.

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Abstract no 34

Computational Chemistry with Relevance to Protein Science

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Brief description of research area:

Computational prediction of substrates and inhibitors for enzymes.

What we know:

We employ a multitude of *in silico* methodologies to study interactions between ligands and enzymes, focusing on both ligand binding and reactivity at the active site. Ligand binding is investigated with standard (rigid receptor) docking, flexible receptor (induced fit) docking, and hybrid quantum mechanical/molecular mechanics (QM/MM) docking. We augment these approaches with molecular dynamics (MD) simulations to provide realistic treatments of the important aspects of enzyme/ligand dynamics and solvation. We elucidate active site reaction mechanisms using QM/MM and reactive forcefield (ReaxFF) calculations.

What we need:

We are always interested in collaborating with experimentalists, as our computational methodology is a natural complement to experiments: It can be applied prospectively to guide the design of drug candidates and analytically in the rationalization of experimental findings. Through mutual validation, the joint computational/experimental approach offers more robust conclusions than either approach alone.

Abstract no 35

Protein structure through chemical cross-linking

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Brief description of research area:

Our research is concentrated on determination of protein structure and interaction using mass spectrometry. In this regard we are particularly concerned with post-translational modifications, *de novo* sequencing, and overall three-dimensional structure.

What we know:

By using chemical cross-linking combined with high-resolution mass spectrometry we are able to determine the overall folding of larger proteins and to determine protein-protein interactions.

What we need:

We would like to collaborate with researchers who are working with well-defined protein complexes, and who would like to know the structure and interactions of these.

Abstract no 36

Monitoring protein crystallization *in situ* by X-ray powder diffraction

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Brief description of research area:

Precipitates of proteins and pharmaceutical compounds can be characterized and identified by X-ray powder diffraction. The characterization includes identification of different crystal forms, *in situ* monitoring of crystal growth, phase transitions and the rearrangements of different crystal forms using specially designed flow cells. The technique provides control of crystallization processes and enables the user to study the influence of different conditions (pH, salt concentrations etc.).

What we know:

We study the crystallization of pure amino acids, sugars, pharmaceutical compounds as well as proteins. We have expertise in X-ray powder diffraction and design of advanced setups for handling protein crystals as well as *in situ* experiments, and handling crystalline slurries in flow cells.

What we need:

More systems, where the crystallization process (kinetics, nucleation, isotropic as well as anisotropic growth) should be studied. At present, the time frame of the crystallization is typically from 1 h to 24 h.

- Pharmaceutical compounds.
- Proteins in relatively larger amounts (minimum 2 ml crystallization solution), which can be precipitated in a controlled way
- Other biological compounds which can be crystallized

Abstract no 37

Development of novel biomaterials from *de novo* designed peptides

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Brief description of research area:

The central aspect of the project presented is the experimental characterization of peptides, which have been *de novo* designed to assemble into fibrils. Proof of successful design and an understanding of the structure formation and mechanism of assembly will enable us to improve the design towards a robust and controllable biomaterial.

What we know:

De novo protein design of both monomer structure and assembly; low-resolution biophysical characterization of conformation and dynamics, such as optical spectroscopy (CD, fluorescence), vibrational spectroscopy (in collaboration), analytical ultracentrifugation, light scattering, electron microscopy; high-resolution structural investigation of fibrils with X-ray fiber diffraction (in collaboration); study of the structure of peptides in solution, structural transitions, association reactions

What we need:

In-depth analysis of electron microscopy data (statistics, image reconstruction)

Abstract no 38

The Intrinsically Disordered Ribonucleotide Reductase Inhibitors Spd1 and Spd2

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Brief description of research area:

The stability of our genetic system is highly dependent on specific proteins operating in tightly controlled settings. When this control is lost, it leads to mutations that ultimately may lead to disease development such as neurodegeneration, cancers or growth defects. In eukaryotic cells one route to minimize the rate of mutation in DNA is to maintain the correct levels of deoxynucleotide triphosphates (dNTPs) that are the building blocks for DNA[1]. This is achieved through stringent regulation of the enzyme Ribonucleotide Reductase (RNR). In *S. pombe* RNR is inhibited by two low molecular weight proteins, Spd1 and Spd2([2] and unpublished data). When the cell initiates DNA replication, Spd1 and Spd2 are degraded leading to RNR activity and hence production of dNTPs for DNA synthesis. It has been shown that degradation of Spd1 is dependent on Spd1 binding to proliferating cell nuclear antigen (PCNA)[3]. However, the structural details and the thermodynamic parameters of the interaction have not been investigated. We have characterised both Spd1 and Spd2 and investigated the structural details of their interaction with PCNA. Investigation of the interaction between the RNR inhibitors and PCNA at a molecular level will provide us with a better understanding of how the cell regulates degradation of these proteins and hence how it regulates RNR activity to maintain a correct level of dNTPs to minimize the mutation rate.

What we know:

We have used Circular Dichroism and Nuclear Magnetic Resonance (NMR) spectroscopy to show that Spd1 and Spd2 are intrinsically disordered proteins and used NMR to identify regions containing transient secondary structure in both Spd1 and Spd2. We have also confirmed the interaction between Spd1 and PCNA and found that Spd2 also interacts with PCNA. Furthermore, we have characterized these interactions at a residue specific level and found that the PCNA interaction site in both Spd1 and Spd2 are located in a conserved region known as the HUG domain which forms a transient α-helix in both Spd1 and Spd2.

What we need:

To progress, we would like to measure the affinity between Spd1 and PCNA as well as Spd2 and PCNA. We would also like to identify the Spd1 and Spd2 interaction site on PCNA. This is not possible using NMR due to the size limitation of the technique. Furthermore, we are also highly interested in identifying the mammalian homolog to Spd1 using various bioinformatics tools.

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Abstract no 39

Real-time monitoring of cellular dynamics by electrochemical impedance spectroscopy

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Brief description of research area:

Electrochemical Impedance Spectroscopy (EIS) is a fundamental analytical technique for the investigation of the electrical properties of biological materials according to their physiological and morphological changes. This technique is a powerful, non-invasive and widely used technique for monitoring cell or tissue cultures and drug screening. Although EIS measurements are simple in principle, they are often complicated in practice. The performance of a precise and reproducible impedance investigation strongly depends on a correctly organized experimental set up, experimental conditions and measuring parameters.

What we know:

In order to optimize any electrochemical impedance spectroscopy measurement system, many are the critical parameters that need to be investigated. Cell adhesion and proliferation can be monitored in real-time using EIS and a precise correlation between the impedance values and the number of cells attached on the surface can be established. The evolution and magnitude of the impedance can be used in order to distinguish different cell lines (HeLa, hVM1,PC12) by size, doubling time, adhesion properties and morphology. Moreover, the kinetics of drug-induced cell death and drug release can be investigated coupling this technique with the well know biophysical ones.
All results can be confirmed by real-time microscopy visualization.

What we need:

Surface functionalization for cell patterning: we want to control the cell-surface interactions to drive the attachment and spreading of the cell population in a specific areas of the microchip.
In particular chemical modification of silicon nitride, silicon dioxide and gold surfaces to facilitate the covalent immobilization of Laminin and other proteins.

Abstract no 40

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

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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 41

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 42

EPR of metalloproteins exemplified by EPR investigations of Cu-insulin

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[1]DTU Chemistry

Brief description of research area:

Electron paramagnetic resonance (EPR or ESR) is a very sensitive method that uses the magnetic response of paramagnetic transition metals to gain information about the metal center and the immediate surroundings in e.g. metalloproteins. Especially copper and iron biomolecules can be investigated this way.
The technique is non-destructive and can be used in solid and liquid phases to determine:

- Coordinated metal ions vs. free (hydrated) metal ions in metalloproteins.
- Coordination number and symmetry of metal sites.
- Quantification of metal sites.

The spectra of copper-insulin will be presented showing

- The crystallographically observed trigonal copper site is time-averaged over tetragonal copper sites.
- EXAFS and EPR agree on the coordination environment around copper whereas X-ray diffraction shows evidence of degradation (photoreduction) by exposure to X-rays even at 20 K and in a saccharose matrix.

What we know:

DTU Chemistry has a Bruker EMX EPR instrument able to measure at both X-band and Q-band.
We are able to simulate the spectra by using computer simulations by theoretical spin Hamiltonian models and extract model parameters.

What we need:

We are interested in all projects with copper containing biomolecules.

Abstract no 43

Characterization of hyperthermopilic enzyme estA

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Brief description of research area:
Characterization of hyperthermopilic enzyme estA by NMR methods.

What we know:
Lysines have been methylated on surface. Structure has been solved by X-ray crystallography.

What we need:
Characterization of thermophile proteins by calorimetry. Expression and protein $^{13}C/^{15}N$ labeling in archea S*ulfolobus* S*olfataricus*. Methods for NMR assignment of dimer protein, where monomer is ~30 kDa and at elevated temperature, ~ 50-70 C.

Abstract no 44

Structural determination of protein complexes from fiber diffraction data using Rosetta Macromolecular Modeling Suite

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Brief description of research area:

Many biologically important protein complexes have a natural tendency to form filaments. This propensity limits or even hinders their crystallization and consequently high-resolution structure determination by means of X-Ray Crystallography. Fiber diffraction is therefore the only practical method of structure determination at the molecular level for these complexes.
Processing of fiber diffraction data is generally more difficult than for single-crystal patterns and requires specialized software (other then routinely used in X-Ray Crystallography). There are a few programs available that allow processing of fiber diffraction patterns and only one that perform a structural refinement. Thus, there is still plenty of room for exploring structural determination by means of fiber diffraction.

What we know:

We know how to develop and apply a method to generate high-resolution, all-atom protein complexes models from fiber diffraction data using Rosetta macromolecular modeling suite. We also know how to write an efficient algorithm using General Purpose Graphical Processor Units (GPGPUs). We have also developed a cross-validation method to overcome over-fitting with experimental data problem.

What we need:

Input from experimentalist that use or used fiber diffraction technique to characterize their systems both form theoretical and practical standpoints.

Abstract no 45

Chemo-enzymatic synthesis of glycosidase-resistant glycolipid analogs

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Brief description of research area:

Glycosphingolipids (GSLs) play numerous roles in cell surface biological recognition events. Analysis of GSLs is conventionally carried out using TLC, HPLC, and Mass-spectrometry. However, these techniques lack sensitivity and are not always quantitative. We are developing methods for profiling GSL metabolism by use of fluorescently-labeled glycolipid analogs. These GSL analogs are taken into cells and metabolic profiles can be established using capillary electrophoresis with laser-induced fluorescence detection. However the saccharides are often hydrolyzed by intracellular glycosidases, therefore anabolic pathways (sugar extensions) are difficult to monitor.

We are developing chemo-enzymatic methods with glycosyltransferases to produce metabolically stable GSLs with sulfur in the inter-glycosidic linkages as well as in the ring oxygens.

What we know:

Chemo-enzymatic synthesis of fluorescently-labeled glycolipids, Capillary electrophoresis, Glycosidase-resistant glycolipid analogs.

What we need:

In vitro protein refolding to produce additional galactosyltransferases for use in analog synthesis.

Abstract no 46

Thymidine Kinase 1 Regulatory Fine-Tuning Through Tetramer Formation

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Brief description of research area:
Molecular Genetics, DNA metabolism, Enzymology

What we know:
Protein expression and purification, Enzyme kinetics

What we need:
Different methods to explain structure-function relationship, Sub-cellular localization of proteins, Differential expression of genes in various tissues.

List of participants

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