PROTEIN SCIENCE UP-DATE

9th Protein.DTU Workshop

22nd May 2013

Contents

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Wednesday 22 May 2013

Technical University of Denmark
Building 306, Lecture hall 31

Programme

08:20-08:50 **Arrival and breakfast**

Chair: Birte Svensson, DTU Systems Biology

08:50-09:00 **Welcome** by **Egon Bech Hansen**
Head of department, DTU Systems Biology

Chair: Birte Svensson, Professor, DTU Systems Biology

09:00-09:30 **Jens Ulstrup**
Professor, DTU Chemistry
Are single protein molecules electronic conductors, or do we believe it when we see single protein molecules in electron transfer and enzyme action?

Chair: Peter M. H. Heegaard, Professor, DTU Vet

09:30-10:00 **Michael Engelbrecht Nielsen**
Associate professor, DTU Food
Proteins involved in the wound healing process

Chair: Jenny Emnéus, Professor, DTU Nanotech

10:00-10:30 **Claus Helix Nielsen**
External associate professor, DTU Physics
Lipid-protein interactions and biomimetic membranes

10:30-10:50 **Coffee break, biscuits and fruit**

Chair: Günther H. J. Peters, Associate professor, DTU Chemistry

10:50-11:20 **Kasper Planeta Kepp**
Associate professor, DTU Chemistry
Protein evolution from sequence to survival: Myoglobin in deep-divers

Chair: Charlotte Bernhard Madsen, Senior scientist, DTU Food

11:20-11:50 **Lasse Eggers**
Postdoc, DTU Vet
The tell-tale protein – MHC class I binding and complex assembly; it's all about affinity and stability

Chair: Maher Abou Hachem, Associate professor, DTU Systems Biology

11:50-12:20 **Martin Dufva**
Associate professor, DTU Nanotech
Microfluidics methods enabling identification of paracrine or autocrine networks involved in stem cell differentiation

12:20-13:00 **Lunch and poster viewing**

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Chair: Michael Engelbrecht Nielsen, Associate professor, DTU Food

13:00-13:30 **Anne Marie Vinggaard**
Professor, DTU Food
Detection and activation of proteins in endocrine disruption research

Chair: Flemming Jessen, Senior scientist, DTU Food

13:30-14:00 **Jesper V. Olsen**
Professor, Center for Protein Research, University of Copenhagen
Global analysis of cell signaling pathways by quantitative phosphoproteomics

Chair: Christine Finnie, Associate professor, DTU Systems Biology

14:00-14:30 **Winnie Svendsen**
Associate professor, DTU Nanotech
Detection and capturing of proteins in a microfluidic device

14:30-14:50 **Coffee break, biscuits and fruit**

Chair: Pernille Harris, Associate professor, DTU Chemistry

14:50-15:20 **Birthe B. Kragelund**
Associate Professor, Department of Biology, University of Copenhagen
Intrinsic disorder in membrane proteins – a fat chance!

Chair: Kristoffer Almdal, Professor, DTU Nanotech

15:20-15:50 **Maher Abou Hachem**
Associate professor, DTU Systems Biology
Molecular determinants of oligosaccharide metabolism by probiotic bacteria

15:50-17:30 **Poster session and "time for a beer"**

List of abstracts

Abstract no 1

High expression of cellulase by multi-agent mutagenised Trichoderma reesei, 3EMS35 mutant using wheat straw as carbon source

Zia Ullah Khokhar[1, 2], M. Amin Athar[2], Jing Wu[3], Irini Angelidaki[1]

[1]Department of Environmental Engineering-Technical University of Denmark (DTU), Denmark
[2]Institute of Biochemistry and Biotechnology, Punjab University, Lahore, Pakistan
[3]College of Mechanical and Transportation Engineering, China University of Petroleum – Beijing , 102249 China.

Brief description of research area:

One of the main challenges for utilization of lignocellulosic material as a feedstock for bioethanol production is the high cost of commercial cellulolytic enzymes. The objective of this research work was hyper and cheaper on-site production of cellulases. We introduce a new mutagenesis technique, which we call multi-agent mutagenesis (MAM), that allows improvement of fungal strain by using different mutagens one after one. *T. reesei* PCSIR-11 fungus was treated with ultraviolet (UV) radiation, ethidium bromide (EtBr) and lastly with ethyl methane sulfonate (EMS) solution respectively for various concentrations and time intervals. After each treatment cellulase enzyme was produced using untreated wheat straw as a carbon source and cellulytic potencial of enzyme was measured in term of specific activity of CMCase and FPase. After treatment with EtBr signifacant morphological changes in the growth style of fungus, mycelial to yeast phase due to genetic alteration, were also noted. As a result of MAM, *T. reesei* $3EMS_{35}$ mutant was obtained, which produced cellulase with highest specific activity of CMCase and FPase. *T. reesei* $3EMS_{35}$ mutant showed 9.0 and 10.5 fold higher specific activity of CMCase and FPase respectively, as compared to the parent strain *T. reesei* PCSIR-11. We expect this new approach to dramatically reduce the cost of enzymes, and hence make bioethanol production economicaly viable.

What we know:

The increased production of cellulase enzyme and its high specific activity was consequence of the mutations in the genetics of the *T. reesei* $3EMS_{35}$ mutant. The consecutive treatment of fungal strain with UV, EtBr and EMS proved to be effective and cheap mutagenic technology. We achieved objectives by development of *T. reesei* $3EMS_{35}$ mutant strain for hyper production of cellulolytic enzymes for saccharification of delignified wheat straw. Instead of use commercial enzymes, on-site produced microbial cellulases by *T. reesei* $3EMS_{35}$ mutant will save lot of foreign exchange. Our findings have much more impact on enzyme economy for biotechnological applications of microbial cellulases.

What we need:

During bioconversion of lignocellulosic biomass to bioethanol, lignin offers great hindrance in the action of cellulases. Biodeliginifacation of plant biomass with laccases will be the solution of this problem. We need on-site enhanced production of laccases and cellulases by improved fungal strain and their potential use in the same vessel saccharification & fermentation (SVSF) of plant biomass for bioethanol production from both C_6 & C_5 sugars, as well as simultaneous separation of ethanol from fermentation broth. This process will integrate on-site enzymes production, hydrolysis, and fermentation of C_6 and C_5 sugars. Sufficient funding is needed for this project.

Abstract no 2

Are protein and DNA molecules electronic conductors, or do we believe it, when we see single protein and DNA-based molecules in electron transfer and enzyme action?

P. Salvatore[1], K.K. Karlsen[2], A.G. Hansen[1], J. Zhang[1], Q. Chi[1], R.J. Nichols[3], J. Wengel[2], and J. Ulstrup[1]

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The scanning probe microscopies, scanning tunnelling (STM) and atomic force microscopy (AFM) enable addressing molecules on solid surfaces with a degree of detail that even reaches the single molecule directly in aqueous chemical and biological media under electrochemical control (*in situ* STM and AFM). Redox molecules are of particular interest but pose greater challenges than non-redox molecules. *In situ* STM and AFM of biomolecules such as (metallo)proteins and DNA-based molecules pose greater single-molecule challenges but offer intriguing insight in the ways these molecules operate.

Single-molecule bioelectrochemistry (metalloproteins, DNA) requires well-defined (atomically planar) electrode surfaces modified by molecular monolayers (SAMs). Such surfaces have themselves been mapped to sub-molecular resolution and disclose an intriguing variety of local environments. ***Structural*** mapping of redox metalloproteins such as blue copper, heme, and iron-sulfur proteins as well as the metalloenzymes nitrite reductase and laccase were first in focus, but is now followed by single-molecule electron transport and enzyme ***function***. These efforts are being extended to DNA-based molecules. We shall overview some of these recent studies and note some observations, theoretical concepts, and some "puzzles".

Four recent references:

1.J. Zhang, A.M. Kuznetsov, I.G. Medvedev, Q. Chi, T. Albrecht, P.S. Jensen and J. Ulstrup, Chem. Rev. 108 (2008) 2737-2791.
2.J. Zhang, Q. Chi, A.G. Hansen, P. S. Jensen, P. Salvatore and J. Ulstrup, FEBS Letters 586 (2012) 526-535.
3.A.G. Hansen, P. Salvatore, K.K. Karlsen, R.J. Nichols, J. Wengel and J. Ulstrup. Phys Chem Chem Phys 15 (2013) 776-786.
4.P. Salvatore, K.K. Karlsen, A.G. Hansen[1], J. Zhang, R.J. Nichols[3] and J. Ulstrup, J. Am. Chem. Soc. 134 (2012) 19092-19098.

Abstract no 3

Novel oligopeptidase B from *Serratia proteamaculans.*

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It was purified a novel trypsin-like protease (PSP) from the psychrotolerant gram-negative microorganism *Serratia proteamaculans*. PSP formed a tight complex with GroEL chaperonin. A method for dissociating the GroEL–PSP complex was developed. Electrophoretically homogeneous PSP had molecular mass of 78 kDa; the *N*-terminal amino acid sequence 1-10 was determined, and mass-spectral analysis of PSP tryptic peptides was carried out. The enzyme was found to be the previously unknown oligopeptidase B (OpdB).

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.

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.

The substrate specificity of PSP was analyzed using *p*-nitroanilide substrates, and the influence of calcium ions on the enzyme activity was studied. Hydrolysis of oligopeptides by PSP was studied using melittin as the substrate. Optimal conditions for the PSP activity (pH and temperature) have been established. PSP has a number of specific features including an unusual substrate inhibition as well as the drastic influence of calcium ions on its activity. It is assumed that the PSP molecule contains a large hydrophobic substrate-binding site, and significant conformational rearrangements of the enzyme active site are induced by Ca^{2+} binding and by the formation of the enzyme–substrate complex. The temperature characteristics of PSP (high activity at low temperature as well as low apparent temperature optimum (25°C) confirm that PSP is a psychrophilic enzyme.

And the main question is how to get stable truncated variant of PSP.

Abstract no 4

Surface Binding Sites in Carbohydrate Active Enzymes

Darrell Cockburn[1], Casper Wilkens[1], Maher Abou Hachem[1], Birte Svensson[1]
[1]Enzyme and Protein Chemistry, Department of Systems Biology, DTU

Brief description of research area:

We are interested in exploring binding sites located on the catalytic modules of carbohydrate active enzymes. These binding sites are found outside of the active site and in fact can be quite distant from it. While these sites may serve similar roles as carbohydrate binding modules (CBMs), they are different in that they are still part of the catalytic module itself. Additionally, many surface binding site (SBS) containing enzymes additionally possess a CBM, suggesting that their functions are complementary. SBS have been observed in crystal structures of several different enzymes and their importance for catalysis have been confirmed in a few cases. We seek to find how prevalent these sites are in carbohydrate active enzymes by screening a large number of these proteins.

What we know:

We have a great deal of experience in the characterization of enzymatic properties, including their kinetics and binding properties. We have the ability to clone, mutate, express and purify the enzymes in either prokaryotic or yeast expression systems. We measure binding properties using surface plasmon resonance, affinity electrophoresis and isothermal titration calorimetry. We are also experimenting with using carbohydrate microarrays to monitor the binding of our proteins.

What we need:

One of the key problems we face is identifying the location of an unknown binding site after we have detected it in one of our screens. We have considered both theoretical prediction of potential binding site locations as well as substrate protection based labeling schemes, but have not found anything completely satisfactory as of yet. Any suggestions in this regard would be greatly appreciated.

Abstract no 5

Mutational Analysis of a Glycoside Hydrolase Family 62 α-L-arabinofuranosidase from *Aspergillus nidulans* FGSC A4 to Discern the Catalytic Residues and Enzyme Substrate Interactions

Andersen, S.[1]; Wilkens, C.[1]; Cockburn, D.[1]; Abou Hachem, M.[1] and Svensson, B.[1]

[1]Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark

Brief description of research area:

The genome sequence of *Aspergillus nidulans* FGSC A4 revealed an α-L-arabinofuranosidase (AN7908.2), which belongs to the glycoside hydrolase family (GH) 62.
We have shown that AN7908.2 interacts with a wide range of carbohydrates in carbohydrate microarrays and affinity gel electrophoresis, however, enzymatic activity have only been detected on arabinoxylans and arabinans. This suggests that a less specific binding site is present on the enzyme, and we are currently through mutational analysis attempting to locate this binding site.

What we know:

Protein production, mutational analysis, protein-carbohydrate interactions, bioinformatics and protein crystallization.

What we need:

Arabinoxylan and arabinan oligosaccharides.

Abstract no 6

Molecular Interactions of Probiotics with Extracts from Medicinal Plants, Oligosaccharides and Adhesion to Mucin

Hasan Ufuk Celebioglu[1], Kristian Mølhave[2], Susanne Brix Pedersen[3], Sampo Lahtinen[4], Maher Abou Hachem[1], Susanne Jacobsen[1], and Birte Svensson[1]

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[2] Department of Micro- and Nanotechnology, Technical University of Denmark.
[3] Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark.
[4] DuPont, Health & Nutrition, Sokeritehtaantie 20, 02460 Kantvik, Finland.

Brief description of research area:

Probiotics are “live microorganisms which when administrated in adequate amounts confer a health benefit on the host” (FAO/WHO). There are certain criteria to be probiotics: resistance to and colonization of the human gastrointestinal tract (GIT), antimicrobial activity against potential pathogens, and modulation of the immune system. Non-digestible oligosaccharides are energy sources for the microorganisms in the GIT. They can have important health benefits for humans by stimulating growth of beneficial bacteria; therefore, they can be classified as prebiotics. Plants have been used for medicinal purposes, including the treatment and prevention of diseases and illnesses. There are several studies related with many plant species and their beneficial effects on human health, including antioxidant activities, digestive stimulation action, anti-inflammatory, antimicrobial, anti-mutagenic effects and anti-carcinogenic potentials. Medicinal plants possess many biological active compounds such as polyphenols and flavonoids. These compounds have potential to exert important effects on human health and may also stimulate beneficial bacteria in the GIT. It is therefore an aim to explore effects of plant extracts used in alternative medicine on probiotics eventually in conjunction with approved carbohydrate prebiotics in order to identify compounds or ingredients from such plants with health-promoting and perhaps even synergistic properties. In GIT, microorganisms interact with mucus layer and adhesion properties of microorganisms are important for this interaction. Therefore, we want to study the effects of plant extracts and potential prebiotic oligosaccharides on adhesion properties of selected probiotic microorganisms.

What we know:

Moonlighting proteins have been described and may have a function in bacteria-host interactions. In preliminary experiments microscopy of *Lactobacillus* grown on e.g. oligosaccharides shows change in surface morphology. As techniques, our group is expert at proteomics, as well as previous studies about probiotics have been conducted in our group.

What we need:

We are going to study with adhesion properties of probiotics which are grown together with plant extracts and potential prebiotics. This includes cell wall/membrane proteomics, which is very challenging due to the preparation. The adhesion of probiotics to mucus or intestinal cells needs optimization. Collaboration with Kristian Mølhave from DTU Nanotech will allow us to observe the adhesion of microorganisms to mucus or intestinal cells by using Electron microscope. However, preparation of biological samples for electron microscope and measuring the morphological changes needs to be figured out. Human intestinal cell lines will be used in collaboration with Susanne Brix Pedersen from DTU Systems Biology and it also needs some optimization and search about technique that is used for adhesion of microorganisms to the mammalian cells.

Abstract no 7

The crystal structure of a starch debranching enzyme in complex with a protein inhibitor reveals an extremely tight and novel mode of interaction

Marie Sofie Møller,[1,2]* Johanne Mørch Jensen,[1,2] Malene Bech Vester-Christensen,[1] Maher Abou Hachem,[1] Anette Henriksen,[2] and Birte Svensson.[1]

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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] The bifunctional α-amylase/trypsin inhibitor (RBI) from ragi (Indian finger millet) belongs to the cereal-type inhibitor family,[2] and the crystal structure of RBI in complex with α-amylase from yellow meal worm[3] provides insight into the mode of inhibition. 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 α-limit dextrins to fermentable sugars.[5] We solved the crystal structure of the LD:LDI complex to 2.7 Å resolution revealing a novel mode of inhibition distinctly different from that of the RBI interaction with α-amylase, and involving the opposite face of LDI compared to RBI. 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.

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).

1. Juge and Svensson, *J. Sci. Food Agric.* 2006, *86*, 1573.
2. Franco et al., *Eur. J. Biochem.* 2002, *269*, 397.
3. Strobl et al., *Structure* 1998, *6*, 911.
4. MacGregor, *Biochim. Biophys. Acta.* 2004, *1696*, 165.
5. MacGregor et al., *J. Cereal Sci.*, 2000, *31*, 79.

Abstract no 8

Designing knowledge based protein structure potentials

Martin Carlsen[1], Peter Røgen[1]

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Brief description of research area:

Modeling of protein energy potentials with the purpose of increasing the accuracy of protein structures models. Two commonly used strategies are: 1) Models are trained to pick the best structure from an ensemble of structures or 2) structures are refined by energy optimization.

What we know:

Peter Røgen and Patrice Koehl have developed a potential particularly good at finding the best decoy in an ensemble of structures since the potential is trained to correlate to the distance from an experimental (native) structure to deformed structures (decoys). We have tested the model on a variety of distances measures and test sets and found that the model is extraordinarily good at ranking decoys compared to statistical and physical potentials on test sets generated in the same manner as the training set.

What we need:

Our test analysis shows that the performance of the model on test sets generated by other methods is reasonable but we want to improve it. We believe that training on decoy sets based on homology modeling or threading will lead to better results. We are therefore particularly interested in 1) ensembles of near-native protein structures generated for example by homology modeling or threading - or 2) collaboration with experts in automatic homology modeling or threading with the goal of optimizing a potential for this porous.
The last author also offers very fast structural comparison, classification and clustering of protein 3d structures and seek applications.

Abstract no 9

Understanding the structure and dynamics of the CRH1 domain of the human leptin receptor

Zigmantas Toleikis, Esben J. Bjerrum, Birthe B. Kragelund and Kaare Teilum

Structural Biology and NMR Laboratory, Faculty of Science, Department of Biology, University of Copenhagen

Brief description of research area.

We are trying to solve a structure of extracellular part of leptin receptor and understand more details about structure dynamics of this receptor. We are purifying different domains of recombinant human leptin receptor and analyzing those using NMR, Fluorescence and CD spectroscopy methods.

What we know:

The leptin receptor (LR) is a cytokine I class receptor activated by the leptin hormone. LR regulates energy intake and expenditure, feeding and body weight. The extracellular part of the receptor consists of seven domains: two N-terminal cytokine receptor homology (CRH)1 domains, an immunoglobulin-like domain (Ig-like), two CRH2 domains, and two fibronectin type III (FNIII) membrane proximal domains. The Ig-like and CRH2 domains are involved in leptin binding and the high resolution structure of CRH2 bound to a leptin-blocking antibody is known (Carpenter et al.,
2012). The role of CRH1 domain is not very well understood, but mutation in this domain caused the obese phenotype of the rat and the signaling of LR was shown to be partially constitutive. It is interesting that CRH1_2 domain (the second domain of CRH1) has a conserved WSXWS motive the same as CRH2 in the leptin binding domain. We hypothesize, that this motive can be important in the receptor activation as suggested shown for the prolactin receptor (Dagil, el al., 2012). Here we present the solution structure of the CRH1_2 domain. In addition we have compared CD spectra of CRH1_2, CRH2_2 and peptide containing the WSXWS from the two proteins and run short MD simulations of the WSXWS peptides to assess the structural preferences of the Trp residues in this motive

What we need:

We are trying to get more NMR data from CRH2 domain to determine a high resolution structure and understanding more details how leptin receptor interacts with leptin. We would also like to compare structure, stability and dynamics of the domains in CRH1 and CRH2 containing WSXWS motive.

Abstract no 10

Glycoproteome analysis of wheat albumins by ZIC-cotton HILIC

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Abstract

Hydrophilic liquid chromatography (HILIC) has been used extensively for glycopeptides enrichment by several different types of HILIC material. Here, a quick and easy strategy to enrich glycopeptides was persued using a combination of two HILIC materials: ZIC-HILIC (Zwitterionic Hydrophilic Interaction Liquid Chromatography) and Cotton for enrichment in both ion-pairing and non ion-pairing mode. With this approach together with site-specific glycosylation labeling and LC-MS/MS, 46 different glycosylation sites were assigned for 40 albumin proteins from wheat grains. Most of identified glycoproteins from wheat albumins are homologous to defense related proteins in plant and food allergens. This global glycosylation analysis provided more information on wheat flour albumin components for future study to improve understanding on protein structures and properties related to the defense mechanism and immunogenicity. Moreover, this study may also provide targets for further study on wheat beer quality.

What we know:

Method for glycopeptide enrichment and identified glycoproteins in wheat and barley

What we need:

Applications/computational analysis for further characterization of glycoproteins

Abstract no 11

Protein purification and characterization at the CHO cell line engineering research section at the NNF Center for Biosustainability

Stefan Kol

Many biological therapeutics are produced as recombinant glycoproteins in CHO cells. The glycosylation of glycoprotein therapeutics is important for their biological functions and therapeutic efficacy. CHO cells produce rather simple glycosylation patterns on recombinantly expressed proteins, which are generally acceptable for therapeutic use in man. There are many exciting opportunities to custom design the glycosylation capacity of CHO cells to improve product homogeneity, to improve specific properties of glycoprotein therapeutics, and to expand the applications of recombinant therapeutics.
At present, a high-throughput CHO cell strain construction pipeline is being set up, wherein a panel of human therapeutic proteins will be expressed. These proteins will need to be purified on a regular basis (~50/month) and facilities that can handle this are being established. Besides purification, part of the analysis will be performed here as well. On the long run, we aim to establish a protein purification facility for the whole Center for Biosustainability.

Abstract no 12

The protein composition of equine wound fluid collected by microdialysis and the potential for mapping of the inflammatory response

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[4] Department of Animal Health, Welfare and Nutrition, Faculty of Agricultural Sciences, Aarhus University, Aarhus, Denmark

Brief description of research area:

Healing of traumatic wounds on the equine distal limb is often complicated by formation of exuberant granulation tissue. Existing research demonstrates a reduced influx of polymorph nuclear cells to the wound bed on the legs. This could indicate an attenuated inflammatory response. The aim of this study was to map the proteins presented in wound fluid from normal and aberrant healing equine wounds and determine if wound fluid is suitable for investigation of the inflammatory response.
Large-pore microdialysis technique was used to collect wound fluid from experimental wounds on the body and the leg of a horse. Eight wound fluid samples collected regularly over a 22-day healing period were analyzed by mass spectrometry (LC MS/MS). Data was searched against an equine database and classified using the gene ontology principles.

What we know:

Wound fluid is suitable for mass spec. analysis of the inflammatory response, especially the acute phase response, during wound healing. These findings provide the basis for the design of a targeted quantitative study for comparing normal and aberrant equine wound healing. This will be done using QconCAT vector technology.

What we need:

Collaborators for two post.doc. applications within the area of equine wound healing.

Abstract no 13

Collection of Wound Fluids from Horses using Microdialysis

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[3] Department of Clinical Medicine, Aalborg University, DK-9000 Aalborg, Denmark
[4] Department of Animal Health, Welfare and Nutrition, Faculty of Agricultural Sciences, Aarhus University, Aarhus, Denmark

Brief description of research area:

Collection of sample material for wound healing research in experimental animals is commonly obtained through biopsies. Though, biopsy collection is an invasive procedure and consequently triggers an inflammatory response. Therefore, wounds should only be biopsied once to display the undisturbed, natural healing process. This necessitates the creation of a wound for every required collection time-point. To limit the number of wounds created on each experimental animal, a new method that allows repeated collection from wounds was sought.
Microdialysis allowed the collection of wound fluid samples for research purposes in a less invasive way than biopsies. This method also allowed repeated sampling from every wound and the obtained sample material was sufficient in amount for metabolite and protein analysis.

What we know:

Microdialysis is a minimally invasive method for sampling of compounds from the extracellular fluid, where a small probe is inserted into the target tissue and flux of solutes into the probe occurs by simple diffusion. The recovered dialysate reflects changes in the composition of the extracellular water phase. Sample collection can be continued for several hours.

What we need:

Collaborators for two post.doc. applications within the area of equine wound healing.

Abstract no 14

Characterization of a novel β-glucuronosyltransferase from *Arabidopsis* involved in the glycosylation of type II arabinogalactan polysaccharides

Adiphol Dilokpimol[1], Eva Knoch[1], Theodora Tryfona[2], Christian P. Poulsen[1], Jesper Harholt[1], Bent L. Petersen[1], Peter Ulvskov[1], Masood Z. Hadi[3], Paul Dupree[2], and Naomi Geshi[1]

[1]Section for Plant Glycobiology, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Denmark, [2]Department of Biochemistry, University Of Cambridge, United Kingdom, [3]Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, USA

Brief description of research area:

Plant cell wall biosynthesis and regulation – Naomi Geshi group belongs to Section for Plant Glycobiology, Department of Plant and Environmental Sciences, University of Copenhagen. We are currently focusing mainly on (a) Discovery of novel glycosyltransferases (GTs) and other plant cell wall modifying enzymes involved in arabinogalactan-protein (AGP) and type II AG biosynthesis, and their applications, (b) Protein-protein interactions of GTs and the nucleotide-sugar transporters, and (c) Recombination production of functional polysaccharide: Gum arabic variants.

In this poster, we present biochemical characterization of a novel β-glucuronosyltransferase from Arabidopsis. This enzyme belongs to GT14 in Carbohydrate Active Enzyme database and has a topology of type II membrane protein. We expressed soluble catalytic domain of the GT in *Pichia pastoris* and characterized the encoding biochemical activity *in vitro*. We present evidence to show that the GT possesses a glucuronosyltransferase transferring GlcA to the terminal Gal of β-1,6-galactan side chain of type II AG via β-glycosidic linkage. This is the first report for biochemical characterization of a plant enzyme in family GT14 which possesses GlcAT activity involved in biosynthesis of type II AG.

What we know:

- Recombination production of plant enzymes/protein using plant, yeast and bacteria expression systems.
- Enzyme characterization and analysis of carbohydrates and plant cell walls using, e.g., conventional enzyme activity assay, HPLC, HPAEC.
- *In vivo* plant protein localization and protein-protein interaction study using confocal microscopy with Förster/Fluorescence resonance energy transfer (FRET) technique.
- Plant molecular biology

What we need:

- Experts in analysis of emulsification properties and surface tension of modified plant cell wall materials as well as other related-material sciences
- Experts in immune-regulating cells and immunomodulatory
- Supply for specific donor and acceptor substrates for glycosyltransferases
- X-ray crystallography of membrane protein complex
- Proteomic analysis of post-translational modifications

Abstract no 15

Optimal estimation of diffusion coefficients from single-particle trajectories

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Brief description of research area:

What we do: Theoretical and statistical analysis and modeling of stochastic data and systems in biophysics, molecular and cell biology, and nanotechnology.

What we know:

We present a simple, unbiased, and practically optimal covariance-based estimator (CVE) of diffusion coefficients from time-lapse recorded trajectories of diffusing particles. The estimator is far superior to commonly used methods based on measured mean squared displacements and even outperforms the analytically intractable and computationally more demanding maximum likelihood estimator (MLE) for relevant parameter ranges. We extend the CVE along with the MLE to the case of diffusion on a fluctuating substrate, e.g., DNA. We apply the methods to hOGG1 proteins diffusing on flow-stretched DNA, a fluctuating substrate, and show that diffusion coefficients are severely overestimated if substrate fluctuations are not accounted for. We find a two-state protein kinetics in data that revealed only simple and too fast diffusion in an earlier analysis based on the mean squared displacement.

What we need:

We are always looking for interesting projects and collaborations.

Abstract no 16

A meta-proteomics approach to explore the barley-microbe interface - Profiling the colonizing fungal community, xylanolytic activities, and the surface associated proteins of barley grains

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Research area:

Cereals like barley naturally produce endo-β-1,4-xylanases for modification of cell walls during seed development and germination (1,2). In addition, barley grains also contain microbial xylanases produced by microorganisms populating the surface of the kernels. The colonizing microorganisms comprise of a diverse collection of bacteria, actinomycetes and fungi. However, microbial xylanase activity is often significantly inhibited by the presence of proteinaceous xylanase inhibitors, i.e. TAXI, XIP and TLXI (3). In a similar manner, the efficiency of the regularly applied microbial xylanases in the cereal-processing industry to improve the product quality is significantly compromised due to the presence of these xylanase inhibitors.

What we know and need:

Xylanase activity measurements on an array of different barley cultivars suggest that there is considerable inter-cultivar variation in the level of both microbial and endogenous xylanase activities, as well as xylanase inhibitor levels. Moreover, harvest year/environment had a significant effect on both the endogenous and microbial xylanases, as well as xylanase inhibition activities. Application of gel-based proteomics (2D-gel electrophoresis, MALDI-TOF-TOF mass spectrometry) coupled with immunoblotting enabled detection of the different types and multiple isoforms of xylanase inhibitors (XIP, TAXI and TLXI). Additional experiments will elucidate impact of genotype, environment and their interaction on the distribution and variation of xylanase activities and inhibition levels.
The plant-microbe interface is under investigation, where we have initiated to profile and characterize the indigenous fungal community and the surface-associated proteins of barley grains in collaboration with Prof. Jens C. Frisvad (CMB-DTU). Initial experiments have been carried out to map the secretomes of selected cereal fungi grown on barley flour or arabinoxylan as substrate, which will provide additional insight into the plant-microbe interaction. More research is required to investigate the metabolite profiles of the isolated cereal fungi.

This work is supported by the Danish Directorate for Food, Fisheries and Agri Business (DFFE), Technical University of Denmark (DTU), and the Danish Center for Advanced Food Studies.

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Abstract no 17

Proteome analysis of *in vitro* maintained barley aleurone layer after tunicamycin and heat shock treatments

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Research area and objectives:

The cereal aleurone layer (AL) plays an essential role in germination by synthesizing and secreting enzymes that hydrolyse the starchy endosperm in response to gibberelic acid produced by the embryo (Finnie et al. 2011). The barley AL can be isolated from the other seed tissues and maintained in culture, allowing the in vitro study of intracellular and secreted protein patterns. In the present work, we analysed the effect of the antibiotic tunicamycin (as inhibitor of the first enzyme in the N-glycosylation pathway) (Luczak et al. 2008) and the heat shock treatment (as causing selective suppression of secretory protein synthesis) (Shaw et al. 2003) in the secreted and intracellular proteomes of gibberelic acid-treated ALs.

What we know:

Proteins reporting changes in their abundance after tunicamycin and/or heat treatments were identified by MALDI-TOF-TOF MS, and grouped into clusters according to the similarity of their expression profiles. The significance of these clusters will be eventually discussed. These results will provide the basis for in-depth studies of the mechanisms involved in protein secretion during barley seed germination.

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Abstract no 18

Identification of serum biomarkers with the ability to predict neurodegenerative changes and segment according to diagnosis

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The department of enzyme and protein chemistry, DTU

Alzheimer's disease (AD) is the most common form of dementia, representing 50-70% of all cases. AD is a slowly progressive neurological disease of the brain. The clinical characteristics of AD are decline in memory and cognitive deficits. There are 38 million people suffering from AD and dementia. This number will increase to 115 millions in 2050 due to an ageing population

No curative treatment exists for AD, even though some clinical symptoms can be alleviated. The treatment of AD is hampered by the lack of easily accessible biomarkers, which can detect early neurodegeneration to identify patients at risk of AD and monitor the progression of the disease and treatment efficacy.

At present there are two forms of biomarkers for AD: imaging-based and cerebrospinal fluid biomarkers. However, there are several limitations of both forms of the current biomarkers. The imaging-based biomarkers are limited by the number of scans allowed due to the risk of radiation and also high cost. The cerebrospinal fluid biomarkers require a lumbar puncture to collect the sample, which is an invasive medical procedure. Furthermore, these biomarkers do not allow diagnosis in the early stage of disease and cannot monitor the response to treatment.

AD is characterized by formation of amyloid plaques and neurofibrillary tangles (NFTs) which are caused by posttranslational modifications of neuronal proteins. Enzymatic cleavage is the most studied posttranslational modification in relation to AD.

This project aims to explore the posttranslationally modified neuronal proteins as serological biomarkers of AD with focus on enzyme cleavage sites. Enzymatic cleavage of proteins results in fragments, of which some are likely to be small enough to cross the blood-brain barrier and enter the circulation.

The aim is to identify specific enzyme-generated fragments of neuronal proteins known to be altered in dementia. This will be accomplished by using mass spectrometry. Monoclonal antibodies will be developed towards the identified fragments and these will be used in ELISA assays. The most promising biomarkers will be validated in pre-clinical studies and clinical cohorts.

Abstract no 19

Mutations within regulatory multi-protein assemblies are important in the evolutionary adaptation of *Pseudomonas aeruginosa* to the Cystic Fibrosis environment.

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Brief description of research area:

In the Infection Microbiology Group, we use colonization of the human cystic fibrosis airways by the opportunistic pathogen *Pseudomonas aeruginosa* as a system for studying evolution in a natural environment. A special interest is a highly successful *P. aeruginosa* lineage that has undergone substantial phenotypic changes which correlates with mutations encoding σ factor subunits of the RNA polymerase complex. A part of our research is to relate the observed phenotypic changes to σ factor competition for binding to core RNA polymerase. We are working to detect the molecular effects of the selected regulatory mutations, and to elucidate how the protein-protein interactions within regulatory multi-protein assemblies (i.e. the RNAP holoenzyme) are modified as a result of these mutations.

What we know:

The mutations in σ factor subunits of the RNAP complex are found within regions associated with either binding to the core RNAP or binding to DNA recognition sequences. The phenotypic shifts observed in the successful *P. aeruginosa* lineage are most importantly observed as a shift in mucoidity. As we have observed one σ factor mutation resulting in a phenotypic shift from non-mucoid to mucoid, but a second mutation in a different σ factor reverting the phenotype back to non-mucoid, we believe that a mutation in one σ factor result in an altered affinity for the core RNAP (or the DNA recognition sequence) compared to the WT and that the mutations result in an overall shift in competition between several σ factors for the core RNAP.

What we need:

Our own expertise lies within the study of evolution and physiology of bacterial species, especially *P. aeruginosa*. We use genetic manipulation, biofilm techniques, genome sequencing, bioinformatics, and microarray analyses. We need to express and purify the WT and mutated σ factors. These are over expressed in *E. coli* and tagged for purification. Core RNAP (RNAP complex without σ factor subunit) needs to be purified directly from *P. aeruginosa*. So far, we have purified holoenzyme (σ saturated RNAP), but have experienced difficulties in removing the σ factor. Therefore we would like the opportunity to get in touch with others performing, or having performed, this form of purification.
We will also use a combination of techniques to study the kinetics and stability of the protein assemblies and interactions. Our goal is to do so by using Surface Plasmon Resonance, *In vitro* transcription, Isothermal Titration Calorimetry and Differential Scanning Calorimetry, but other creative and interesting ideas to study these interactions are welcome as well.

Abstract no 20

Mutational analysis of residues involved in binding of substrates in the active site and on the surface of a GH62 α-L-arabinofuranosidase from *Aspergillus nidulans*

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Brief description of research area:

Carbohydrate active enzymes face several challenges when acting upon polymeric substrates. These can include localization to the correct substrate in a complex environment (e.g. a plant cell wall), disentangling a single strand from the rest of the material and in the case of processive enzymes, translating along that strand. Some of these challenges are thought to be met by accessory domains such as carbohydrate binding modules (CBMs). While common, these modules are not universal, with many enzymes that are active on polymers lacking such aids to catalysis. In addition, the presence of CBMs does not preclude other interactions between the catalytic module and the substrate (in addition to the active site). These interactions are referred to as occurring at surface binding sites (SBS).

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 SBSs been characterized and shown to have an impact on the enzyme's functionality. The understanding of these SBS 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 SBS preclude a generalisation and may mask the significance of these sites in catalysis.

Glycoside hydrolase family 62 (GH62), which only comprises α-1,5-L-arabinofuranosidases (ABF), has received little attention and nothing has been reported previously about the binding of substrates within this family. 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). Based on homology models we have located and mutated potential binding sites and compared their kinetics on wheat arabinoxylan, sugar beet L-arabinan, *para*-nitrophenyl-α-L-arabinofuranoside, and wheat arabinoxylan derived oligosaccharides. The mutant's ability to bind to polysaccharides was investigated by AE.

What we need:

We need arabinoxylo-oligosaccharides of various lengths and with different arabinosyl substitutions to measure binding on surface plasmon resonance and isothermal titration calorimetry.

Acknowledgement:

This work is supported by The Danish Natural Science Research Council (FNU) (to BS), by a joint PhD fellowship from the Technical University of Denmark and FNU (to CW) and a Hans Christian Ørsted Post-doctoral fellowship from the Technical University of Denmark (to DC).

Abstract no 21

Using microfluidics to study programmed cell death: A new approach

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Brief description of research area:

This project is focused on the development of a microfluidic tissue culture system with electrochemical and optical detection for monitoring in real time events related to programmed cell death (PCD) in barley aleurone layer. The real time monitoring of PCD related events in the microfluidic platform enables a more deep understanding of the underlying mechanisms of PCD in plants.

What we know:

Microfluidic tissue culture enables *in vitro* experiments to approach *in vivo* conditions. Microfluidics also allow implementation of a wide range of electrochemical or optical assays for online, real-time, parallel analysis of important parameters such as redox activity, O_2 and H_2O_2 concentration, extracellular pH, cell viability and enzyme activity[1,2].

Currently, we are optimising an intracellular whole-cell redox activity assay[3] that detects changes in redox activity in barley aleurone layer during PCD. The assay uses a double mediator-system to electrochemically measure redox activity via changes in the NADP:NADPH ratio. Initial experiments showed that the redox activity changes depending on phytohormone activation or inactivation of aleurone layer metabolism and subsequent PCD. This is similar to H_2O_2 concentration changes observed recently by Ishibashi *et al*[4].
We have also successfully detected PCD induced by phytohormones in barley aleurone layer using a double-fluorescent probe-system also used by Fath *et al*[5].

What we need:

As a next step we'll work on the adaptation of an existing microfluidic system in order to integrate both assays (electrochemical and optical). Furthermore we'll focus on the optimization of an assay for measuring extracellular H_2O_2 in microfluidics, which will be challenging due to the volatile nature of H_2O_2. The starting point for this will be an existing assay employed at EPC for measuring intracellular H_2O_2 concentrations in barley aleurone layer.

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Abstract no 22

Determining the Sub-specificities of GH42 β-Galactosidases from Probiotics

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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 project we aim at understanding the molecular recognition between carbohydrate active enzymes (glycoside hydrolases from family 42) and the yet unidentified substrates containing terminal non-reducing β-D-galactose. 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 23

Proteins and beer: Challenges and opportunities

Tim Hobley[1]

DTU-Fødevareinstituttet

Brief description of research area:

DTU-Brewery conducts research, innovation and education at pilot and lab scale in all areas of beer brewing.

What we know:

Proteins and enzymes are an important constituent of the raw materials used, and they end up in the product and in the waste streams. Some proteins and peptides are desirable, whilst others are unwanted and contribute to haze formation, bitterness or e.g. affect gluten intolerant consumers.

What we need:

We are interested in working with experts knowledgeable in the area of beer enzymes, proteins and peptides and their analysis. We are specifically interested in proteins and peptides contributing to bitterness as well as those responsible for gluten intolerance.

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