Title: Real-time monitoring of stem cell differentiation in brain tissue using microfluidic on-line culture systems

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DTU Nanotech –Department of Micro and Nanotechnology, Technical University of Denmark, Kongens Lyngby

Brief description of research area:

Stem cell therapy to treat Parkinson's disease is under focus. The project aims at transplanting and characterizing neuronal stem cells in the brain region, called Striatum, where they differentiate to dopaminergic neurons. This transplantation and characterization are studied in microfluidic system with electrochemical and fluorescence microscopic detection.

What we know:

In preliminary tests we long-term cultured brain tissues in microfluidic system and we electrochemically detected the dopamine release under stimulation of neuronal stem cells.

What we need:

We need to learn more about fluorescence based detection, which can be used to monitor influx of calcium in the neuronal stem cells during exocytosis event. We also want to use molecular beacon to visualize mRNA appearance when Tyrosine Hydroxylase is synthesized.

2

Title: 3D matrices to study cell migration

Sofie Folke Bruun[1], Gregers Jungersen[1], Jenny Emnéus[2], Anders Wolff[2].

[1]DTU Vet, [2]DTU Nanotech

Brief description of research area:

Part of the project aims at studying cell-cell interaction and migration of immune cells. It is an interdisciplinary project combining immunological knowledge with nanotechnological solutions. The work includes different microscopy techniques, 3D matrix gels and freshly harvested mammalian cells.

What we know:

The project includes people from three different groups at DTU - Adaptive Immunology and Parasitology (Vet), Bioanalytics group (Nanotech), and BioLabChip group (Nanotech). Therefore our combined expertise spans immunological techniques as well as microfluidics. In the project we aim to use a simple protein matrix to facilitate cell migration and interaction for immunological studies.

What we need:

To have a chat with people that has experience with:

- Protein immobilization techniques for creating 3D extracellular matrices
- Long term storage of protein matrices
- Confocal microscopy or other techniques for visualizing real time cell migration

3

Regulation of annexins following tissue damage as coursed by infection – investigated by 2-dimensional gel electrophoresis

Tune Wulff and Michael Engelbrecht Nielsen

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Within the Biological Quality Research group we are trying to understand how biological processes in the living animal (using fish as a model) are reflected in the animal post mortem. We are therefore investigating different incidents know to affect the fish when raised in aquaculture. These include hypoxia, crowding, diseases and tissue damage. The aim is to be able to link biological processes with the eating quality of the fish, as the basis for an improved utilization of fish for production.

Using gel-based (2 dimensional gel electrophoresis) proteomics we have investigated several different incident. The workflow as shown on the poster includes: uni- and multivariate data-analysis, protein identification, followed by investigation of functional relationships between identified proteins using bioinformatics tools like Panther, String and Cytoscape.

Investigating tissue damage we were able to uncover a network of interacting proteins, in which three different members of the annexin family, namely annexin- IV, V and VI, were central proteins. While closely examining this network it was also clear that these three proteins have interaction partners normally associated with the immune system (EGF and Chemokine-receptor 5), but also with proteins involved in regeneration and modification of the cytoskeleton (Hsp70 and Cofilin-2).

Wanting to apply gel free method to our field of interest, we are seeking expert opinion on optimal design and workflow for quantitative and targeted studies, including considerations before initiating the experiment.

4

Title: Polymer Micro- and Nanotechnology for On-Chip Cell Selection

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Brief description of research area:

The project aims to improve the efficiency and reduce the costs associated with cancer immunotherapy, by developing a polymer device being able to capture, culture and subsequently release specific immune cells. Micro- and nanostructured polymer surfaces coated with cell-binding proteins, allows specific capture and controlled cell adhesion to the surface. Injection molding is used to simultaneously shape and transfer biofunctional molecules from the surface of a mold to polymer chips. The captured cells (monocytes) are cultured in a microfluidic environment, with automated delivery of nutrients and signalling molecules that induce maturation to dendritic cells, which are the therapeutic candidate.

What we know:

- How to make protein micro-patterns through ink-jet printing
- Measure cell adhesion to protein-coated surfaces

What we need:

- Measure protein concentration on substrate surface quantitatively, with micrometer spatial resolution
- Characterize orientation of surface immobilized antibodies

Title: Functional Electrospun Nano/microstructures Made From Fish Proteins As a New Drug Delivery System

Karen B. Stephansen[1], Flemming Jessen[1], Ioannis S. Chronakis[1].

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Brief description of research area:

Electrospinning is an emerging technique where a (bio)polymer solution is formed into ultrafine nanofibers and other nano/microstructures. The polymer solution is pumped out of a syringe, and by applying a strong electric field between the syringe and a collector plate, a so called Taylor cone is formed. When a critical voltage is exceeded, the polymer solution is ejected from the cone towards the collector plate. On the way from the syringe to the collector plate, the solvent evaporates, leaving behind a thin nanofiber. Nanofibers have many applications, such as tissue engineering, wound dressing, and drug delivery – and this is only within the biomedical research area. As the electrospinning technique evolves, fibers become more advanced and consequently the range of applications increases.

A wide range of different polymers and biopolymers can be used for electrospinning; such as polyvinyl alcohol and poly(ethylene oxide). Proteins such as collagen and gelatine have been successfully spun, however in general proteins and peptides are very poor studied materials within the electrospinning context.

We develop micro/nanofibers, using the electrospinning technique, with fish proteins and peptides as materials. The aim is to use the micro/nanofibers as a new oral drug delivery system.

What we know:

Centrifuged tissue fluid (CTF) from cod constituting a mixture of cytoplasmic proteins was spun into nano and microfibers using an in house electrospinning setup. To investigate the influence of small molecular weight compounds on fiber morphology, the CTF was dialyzed eliminating compounds with a molecular weight below 6-8 kDa. The morphology of the fibers was investigated using scanning electron microscopy. Hexafluoroisopropanol (HFIP) was used as solvent. To examine what effect HFIP had on the CTF proteins, CTF dissolved in water and HFIP respectively were measured with circular dicroism. Additionally CTF dissolved in water or HFIP and CTF fibers were analyzed with differential scanning calorimetric to investigate potential differences in interactions under the different conditions.

What we need:

-

Title: Mucin-terminated surfaces as model systems to investigate the interfacial properties of epithelium

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[1]*Materials and Surface Engineering, Department of Mechanical Engineering, Technical University of Denmark*

Brief description of research area:

Seunghwan Lee's Nanotribology and Biotribology Group at DTU Mechanical Engineering is running a research program focusing on mucins and mucus gels (ERC Starting Grant 2010, Soft, Slimy, and Sliding Interfaces: Biotribology of mucins and mucus gels). This project is initiated to understand the lubricating properties of mucins and mucus gels with different origins, and yet it requires a broad range of research activities, from isolation and purification of glycoproteins, identification of molecular composition and structure of mucins, and surface biophysical/biochemical properties of mucin layers. Ultimately, it is an important goal of this project to generate mucin-terminated surfaces to model epithelium, in order to investigate a broad range of interfacial properties, including lectin-glycan interaction, mucoadhesion, bacterial inoculation, drug delivery, as well as lubricating properties.

What we know and what we can do:

Various types of mucus from porcine internal organs are readily available, and methodology to isolate mucin molecules from mucus is being developed. Mucin-terminated surfaces via spontaneous adsorption, self-assembly, and chemical bonding are being developed. Experimental facilities for optical spectroscopy (CD, fluorescence, absorbance), adsorption of molecules on surfaces (OWLS, QCM-D, goniometry), surface chemical modification (Plasma cleaner/sterilizer, UV/Ozone cleaner), tribology (pin on disk tribometry, MTM), nano-scale surface force (Atomic Force Microscopy), ELLA and ELISA assays, are fully available.

What we need:

Expertise/facilities in mass spectrometric identification of carbohydrate species in complex glycoproteins are needed. To extend the application of mucin-terminated surfaces, collaboration with research groups with expertise/experiences in drug delivery, polymers, bacteria, surface forces, cancers, and relevant biomedical engineering, are sought after.

Title: Hypoxia as a cause of excessive granulation tissue formation in secondary healing equine wounds

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Brief description of research area:

Traumatic wounds on the distal limb often afflict horses. Due to contamination, excessive skin tension and massive tissue losses, limb wounds often have to heal by secondary intention. This often results in formation of exuberant granulation tissue (EGT), a condition which despite sharing many similarities with the human conditions keloid and hypertrophic scarring forms a unique pathophysiological entity unknown from other mammals.

Many factors implied in the pathogenesis of exuberant granulation tissue formation seem to be related to hypoxia in the wound bed. Several EGT studies have found persistent chronic inflammation in the wound bed, a condition that is known to be a critical feature in tumor development. The formation of excessive granulation tissue resembles tumor growth in its uncontrolled behavior, and in tumor development presence of hypoxia has been shown to increase tumor size.

We have collected equine wound fluids through microdialysis catheters and taken biopsies from experimentally made wounds of six horses, both from body wounds (control) and limb wounds (sick).The obtained biopsies and wound fluids will be analyzed using LC MS/MS mass spectrometry, ELISA and qRT-PCR to identify and quantify expression levels of certain hypoxia and chronic inflammation related cytokines, growth factors and other relevant biomolecules. This approach should lead to a better understanding of the network of events leading to formation of ETG. Moreover, it may enable protein expression profiles for wounds healing normally and wounds healing with formation of EGT to be established.

What we know:

- The microdialysis technique
- ELISA
- Experimental animal models

What we need:

- Expertise within the field of detecting low abundant molecules in blood, tissue homogenates and microdialysates using LC MS/MS mass spectrometry
- Expertise in development and use of QconCAT
- Expertise within the field of analyzing proteomic data, including the use of QconCAT data for quantification of protein levels

Exploring the Substrate Specificity of a Glycoside Hydrolase Family 62 α-L-arabinofuranosidase from *Aspergillus nidulans* FGSC A4

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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.

Title: Interactions of multiple high-temperature events on antioxidant enzyme activities in sub-cellular leaf fractions of wheat

Xiao Wang[1, 2,3], Bernd Wollenweber[1], Dong Jiang[2], Susanne Jacobsen[3]
[1]Aarhus University, Denmark [2]Nanjing Agricultural University, China; [3]Technical University of Denmark

Brief description of research area:

Plant stress physiology, molecular biology

What we know:

Knowledge about plant stress physiology, analysis including gas exchange, light use efficiency, reactive oxygen species and related enzymes in sub-cellular, gene expression, a little about proteomics

What we need:

More knowledge about plant proteomics analysis methods event at the sub-cellular level;

How to use proteomics to get useful information combined with plant physiology, in order to relate structure to function.

Multi-disciplinary approaches to prepare for climate change, the biggest challenge in the future**.**

Prebiotic galacto-oligosaccharide utilization by *Lactobacillus acidophilus* NCFM. Establishment of a methodological platform for protein discovery

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Probiotic bacteria have been shown to positively modulate human chronic bowel disorders, immunity and life-style diseases. Carbohydrate utilization has been identified as an important determinant of probiotic action. Utilization of non-digestible oligosaccharides (prebiotics) can selectively stimulate probiotics in the gastrointestinal tract. However, only few carbohydrates are classified as prebiotics mainly due to limited experimental knowledge of *in vivo* studies and molecular understanding of their selective utilization.
The aim of the work was to use an array of omics technologies to provide a comprehensive and molecular understanding of prebiotic utilization, represented by the commercial *Lactobacillus acidophilus* NCFM.
Here we demonstrate the versatility of this approach by discerning the molecular basis of uptake and catabolism of galacto-oligosaccharides containing α- or β-linkages. Transcriptional analysis led to the discovery of the first β-galacto-oligosaccharide transporter within the *Lactobacillus* genus (1) which displayed extensive substrate specificity with respect to oligosaccharide size and composition. This work was integrated with the proteomic reference map and DIGE-proteomics of *L. acidophilus* NCFM (2), to reveal the catabolic pathways of potential galactoside prebiotics and identified the upregulated specific glycoside hydrolases of GH families 2, 42. Additionally transcriptional and functional genomics analysis of α-galacto-oligosaccharides identified a key catabolic α-galactosidase which was biochemically and structurally characterized, highlighting the specificity of this enzyme towards raffinose family oligosaccharides. Sequence motifs and other structural elements important for the molecular architecture were identified allowing us to propose a subdivision of GH36 (3) which differentiated sub-specificities within the family.
This work provides a more detailed view of key mechanisms underlying utilization of prebiotic galactosides within probiotic lactobacilli.

This work is supported by grants from Danish Council for Strategic Research, Committee for Health, Food and Welfare, Danish Council for Independent Research Natural Sciences, a HC Ørsted Postdoctoral Fellowship (to AM), PhD stipends from the Technical University of Denmark (to JMA and ME) and Danisco A/S (to JMA).

References: (1) Andersen et al. PNAS (2011) 108: 17785-17790 (2) Majumder et al. Proteomics (2011) 17: 3470–3481 (3) Fredslund et al. JMB (2011) 412: 466–480

Proteomics approach coupled with activity assays to study variability in xylanase and xylanase inhibition activities in different barley varieties

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Research area:
Exogenous enzyme supplementation of animal feeds has been practiced advantageously for the past three decades. Cereals like barley naturally produce endo-β-1,4-xylanases for modification of cell walls during seed development and germination. In addition, barley grains also contain microbial xylanases produced by microorganisms populating the surface of the kernels. However, microbial xylanase activity is often significantly inhibited by the presence of proteinaceous xylanase inhibitors (TAXI, XIP and TLXI).

What we know:
The present project sets out to investigate variability in xylanase (plant vs. microbial) and xylanase inhibition activities in different barley cultivars. This is done by 2D-gel based proteomics approach (2D electrophoresis and mass spectrometry) coupled with immunoblotting and activity assays. Considerable inter-cultivar variation in the level of both xylanase activities as well as at xylanase inhibitor levels was determined. Application of proteomics analyses (2D-gel electrophoresis, MALDI-TOF-TOF mass spectrometry) coupled with 2D-immunoblots enabled detection of the different types and multiple isoforms of xylanase inhibitors.

What we need:
It is of interest to correlate the measured xylanase activities with the commensal microbial community found on the surface of barley grains. Initial experiments have been performed in collaboration with Jens Frisvad (CMB-DTU).

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

Title: Different Behavior of *Lactococcus lactis'* Thioredoxins

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Thioredoxin (Trx) is a small protein contributing in many crucial live aspects of organisms ranging from bacteria to humans. Among its functions belong maintenance of the intracellular reducing environment, oxidative stress response, providing electrons to specific reductases (e.g. ribonucleotide reductase, arsenate reductase, methionine sulfoxide reduxtase...) and also (dis)activation of several transcription factors. The latter one leads to a wide range or pathways influenced by Trx.[1–4] Trx employs its redox-active CXXC motif to reduce protein disulfides by thiol-disulfide exchange during which the target protein becomes reduced while Trx is oxidized. Then it needs to be reduced again by Trx reductase. These proteins are mostly NADPH-dependent (NTR), but also ferredoxin-dependent (FTR) ones exist in chloroplasts and cyanobacteria. There are also glutaredoxins (Grx), glutathione-dependent disulfide reductases, overlapping some functions with Trx in various organisms.[5–7] But this is not the case of *Lactococcus lactis* as well as many Gram-positives.[8,9] *L. lactis* has one NTR (LlTrxB), a Trx (LlTrxA), a Trx-like protein (LlTrxD) and a Grx-like protein (LlNrdH). The latter one is similar to Grx but it was shown to interact with LlTrxB and its function is to provide electrons to NrdEF class Ib ribonucleotide reductase.[10]

LlTrxA possesses a conserved CGPC active site motif while LlTrxD contains CGDC motif and misses for example a conserved aspartate residue suggested to be involved in the catalytic mechanism. In total, LlTrxD shows 28% identity and 36% similarity to LlTrxA. LlTrxA also resembles well-characterized TrxA of *Escherichia* coli in several reactivity aspects (nucleophility, insulin cleavage, redox potential) while LlTrxD does not. The physiological function of LlTrxA is connected with oxidative stress since *ΔtrxA* strain suffers by oxidative stress even at microaerophilic contitions and shows higher sensitivity towards H_2O_2, diamide and paraquat. The function of LlTrxD is not clear, but *ΔtrxD* strain behaves like wild-type in the mentioned cases and it has only shown sensitivity towards arsenate and tellurite so far. However, *ΔtrxAΔtrxD* double mutant shows even more significant growth defects at all conditions.

References:

1. Holmgren, A. Thioredoxin. *Annual review of biochemistry* **54**, 237-71 (1985).

2. Arnér, E.S. & Holmgren, a Physiological functions of thioredoxin and thioredoxin reductase. *European journal of biochemistry / FEBS* **267**, 6102-9 (2000).

3. Collet, J.-F. & Messens, J. Structure, function, and mechanism of thioredoxin proteins. *Antioxidants & redox signaling* **13**, 1205-16 (2010).

4. Antelmann, H. & Helmann, J.D. Thiol-based redox switches and gene regulation. *Antioxidants & redox signaling* **14**, 1049-63 (2011).

5. Holmgren, A. Thioredoxin and glutaredoxin systems. *J. Biol. Chem* **264**, 13963-13966 (1989).

6. Lillig, C.H., Berndt, C. & Holmgren, A. Glutaredoxin systems. *Biochimica et biophysica acta* **1780**, 1304-17 (2008).

7. Holmgren, A. *et al.* Thiol redox control via thioredoxin and glutaredoxin systems. *Biochemical Society Transactions* **33**, 1375–1377 (2005).

8. Fahey, R.C., Brown, W.C., Adams, W.B. & Worsham, M.B. Occurrence of glutathione in bacteria. *Journal of bacteriology* **133**, 1126-9 (1978).

9. Li, Y., Hugenholtz, J., Abee, T. & Molenaar, D. Glutathione protects Lactococcus lactis against oxidative stress. *Applied and environmental microbiology* **69**, 5739 (2003).

10. Jordan, A. *et al.* The ribonucleotide reductase system of Lactococcus lactis. Characterization of an NrdEF enzyme and a new electron transport protein. *The Journal of biological chemistry* **271**, 8779-85 (1996).

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Structural and mutational analysis provide a snapshot into the mechanism and energetics of inhibition of starch α-1,6 debranching enzymes

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Brief description of research area:
The debranching enzyme limit dextrinase (LD) from barley and its endogenous inhibitor (LDI) are the target for my research. LD is the only enzyme which hydrolyse α-1,6-linked branches in α- and β-limit dextrins. Besides limit dextrins, LD hydrolyses pullulan, and to a low extent amylopectin. LD is a part of the concerted action towards mobilising glucose from starch in germinating barley seeds together with α-amylase, β-amylase, and α-glucosidase [1,2].
The techniques I use in my research: Protein structure determination by X-ray crystallography, enzymatic analysis/assays, recombinant protein production/purification, enzyme analysis by rational mutagenesis based on crystal structures, and surface plasmon resonance (SPR).

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 SPR.

What we need:
Branched oligosaccharides – glucose unites connected by α-1,4 glycosidic linkages and α-1,6 linkages at branch points.

1. M. Kristensen et al., Biochim. Biophys. Acta. Protein. Struct. Mol. Enzymol. 1431, 538-546 (1999)
2. R.A. Burton et al., Plant Physiol. 119, 859-871 (1999)

Molecular Interactions in Thioredoxin Systems

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Thioredoxin (Trx) is a protein disulfide reductase in all domains of life. It is well known for providing reducing equivalents to ribonucleotide reductase, which is required for DNA synthesis, but it can reduce a wide range of target disulfides. Our previously determined structure of barley Trx in complex with barley α-amylase/subtilisin inhibitor (BASI) provided detailed information about intermolecular interactions [1]. The structural study has been followed up by a kinetic study including site directed mutants of Trx, which is presented on the poster. It is one of very few cases in which a rate of reduction of a structural disulfide has been determined. The results with the mutants of Trx point at important roles of main chain to main chain interactions as well as electrostatic contacts in target recognition. Trx is specifically reduced by the flavoprotein NTR (NADPH dependent Trx Reductase), and this protein is of course within our research interest. We are characterizing the NTRs from barley and *Lactococcus lactis* in comparison to the more well known NTR from *E.coli*. The 3D structure of barley NTR has been determined in collaboration with the Carlsberg laboratory [2].

Our background in Trx biochemistry might be helpful for others. We can provide expertise in thiol/disulfide chemistry and redox proteomics. For instance, what do you know about the stability (reactivity) of disulfides and thiols in your favourite protein? We are interested in subjecting our proteins to your characterization and *vice versa*. A great number of structures of Trxs and NTRs are available making structure determinations, related to specific questions, feasible by Molecular Replacement. We indeed have such questions but lack at present collaboration with a protein crystallographer.

The Danish Council for Technology and Production Sciences (FTP, grant nr 274-08-0413) is acknowledged for support

[1] K. Maeda, P. Hägglund, C. Finnie, B. Svensson, and A. Henriksen, Structure 14, 1701 (2006)

[2] K. G. Kirkensgaard, P. Hägglund, C. Finnie, B. Svensson and A. Henriksen, Acta Cryst. D65, 932 (2009).

Title: Surface Binding Sites in Carbohydrate Active Enzymes

Darrell Cockburn[1], Casper Wilkens[1], Maher Abou Hachem[1], Birte Svensson[1]

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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, they are different in that they are still part of the catalytic module itself. These sites 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 soon 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.

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Enrichment of glycopeptides from wheat albumin extracts by ZIC®-HILIC

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Brief description of research area:

The study of plant proteomes is important to further the understanding of biological processes and enhance the agronomical and nutritional value of crops and food products. To gain deeper understanding on the proteome level, it is important to characterize post-translational modifications that influence the function, structure, localization, turnover and interaction with other proteins (1,2). For glycoprotein analysis, zwitterionic hydrophilic interaction liquid chromatography (ZIC®-HILIC) has been demonstrated as a useful tool for depletion of hydrophobic peptides and retention of hydrophilic glycopeptides, e.g. from in-solution tryptic digests of human plasma (3,4). The aim of this work is to apply this methodology to screen and identify glycosylated proteins from wheat albumin extracts.

Glycopeptides enriched by ZIC®-HILIC were analyzed by MALDI-TOF MS and the enriched peptides were further subjected to deglycosylation with PNGase A in the presence of ^{18}O labeled water followed by LC-MS/MS analysis. In preliminary experiments, 19 glycosylation sites from 18 matched proteins were detected in wheat albumin extracts.

The program Strategic Scholarships for Frontier Research Network (SFR) of Thailand's C

References

1. Hitchen P.G., Dell A. (2006). Bacterial glycoproteomics. Microbiology 152:1575-1580.
2. Mann M., Jensen O.N. (2003). Proteomic analysis of post-translational modifications. Nature Biotechnology 21:255-261.
3. Hägglund P., Bunkenborg J., Elortza F., Nørregaard O.J., Roepstorff P. (2003). A new strategy for identification of N-glycosylated protein and unambiguous assignment of their glycosylation sites using HILIC enrichment and partial deglycosylation. Journal of Proteome Research 3:556-566.
4. Hägglund, P., Matthiesen, R., Elortza, F., Højrup, P., Roepstorff, P. Jensen, O. N., Bunkenborg, J. (2007) An enzymatic deglycosylation scheme enabling identification of core fucosylated N-glycans and O-glycosylation site mapping of human plasma proteins. Journal of Proteome Research 6:3021-3031