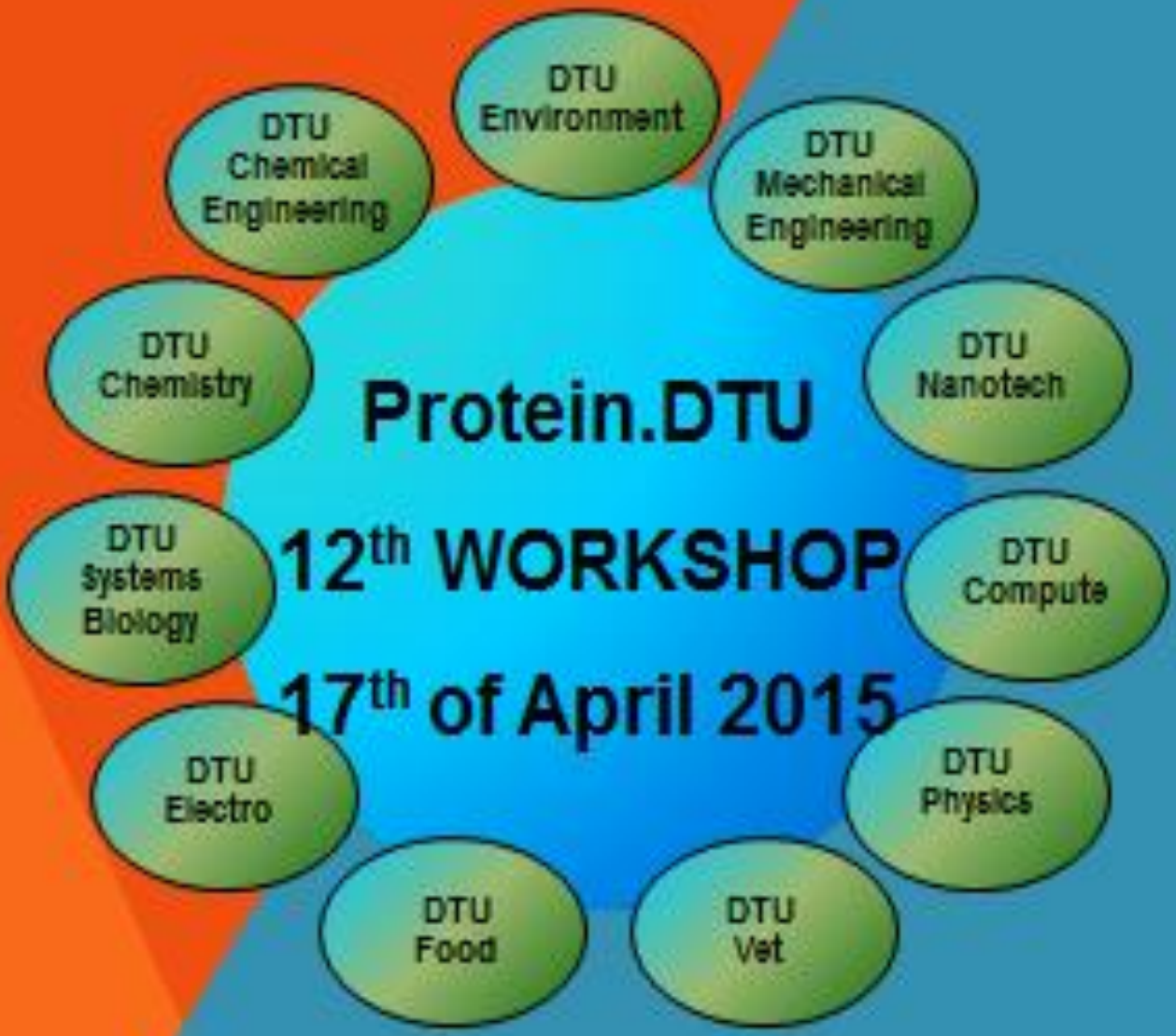
DTU Environment
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12th WORKSHOP
17th of April 2015
DTU Systems Biology
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Protein.DTU

12th Workshop

17th of April

12th Workshop in Protein.DTU
Protein Science across DTU

April 17, 2015
Glassalen, Building 101,
Anker Engelundsvej 1, Kgs.
Lyngby

08:30 - 09:00		**Arrival & Breakfast**
09:00 - 09:10		**Welcome** **Hanne Østergaard Jarmer** (Director, DTU Systems Biology)
09:10 - 09:40		**The role of dendritic cells in controlling intestinal immune responses** **William Agace** (Professor, DTU Vet) Chair: Peter Heegaard (Professor, DTU Vet)
09:40 – 10:10		**Understanding and manipulating the lubricity of mucinous glycoproteins** **Seunghwan Lee** (Associate Professor, DTU Mechanical Engineering) Chair: Maher Abou Hachem (Associate Professor, DTU Systems Biology)
10:10 – 10:40		**Proteins in sensing and separation: a biomimetic perspective** **Claus Helix Nielsen** (Associate Professor, DTU Environment) Chair: Birte Svensson (Professor, DTU Systems Biology)
10:40 – 11:10		**Coffee Break**
11:10 – 12:20	Chairs:	**FLASH PRESENTATIONS - 4 min each** Kristoffer Almdal (Professor, DTU Nanotech) Charlotte B. Madsen (Senior Scientist, DTU Food) Pernille Harris (Associate Professor, DTU Chemistry), Michael Engelbrecht (Associate Professor, DTU Food)
12:20 – 13:30		**Lunch and poster viewing**
13:30 – 14:00		**An introduction to the DTU proteomics platform – the *Lacococcus lactis* thioredoxin system** **Per Hägglund** (Associate Professor, DTU Systems Biology) Chair: Ole Lund (Professor, DTU Systems Biology)

14:00 – 14:30 **Proteins in your DVD player**

Anja Boisen (Professor, DTU Nanotech)
Chair: Kristoffer Almdal (Professor, DTU Nanotech)

14:30 – 15:00 **Functional protein nano-microstructures for bioengineering applications**

Ioannis Chronakis (Senior Scientist, DTU Food)
Chair: Flemming Jessen (Senior Scientist, DTU Food)

15:00 – 16:30 **Poster session, coffee and "time for a beer"**

Chairs: Günther Peters (Associate Professor, DTU Chemistry)
Flemming Jessen (Senior Scientist, DTU Food)
Kristoffer Almdal (Professor, DTU Nanotech)

NO 1

Title: Understanding how added milk protein ingredients interact with other milk components during processing and how this affects final dairy product quality

Guanchen Liu[1], Richard Ipsen[1], Marie Tholstrup Greve[2].

[1] *Section for Dairy, Meat and Plant Product Technology, Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark.*

[2] *Arla Foods Ingredients Group P/S, Sønderupvej 26, 6920 Videbæk, Denmark.*

Brief description of research area:

The project aims at utilizing a number of advanced techniques (surface plasmon resonance (SPR), isothermal titration calorimetry (ITC) and quartz crystal microbalance (QCM)) for characterizing and quantifying interactions between milk protein aggregates (Micro-, and Nanoparticulated whey protein) and inherent milk components (casein micelles, fat globules) in model systems designed to emulate milk and with a gradual increase in complexity. This will then be related to the properties of the aggregates (e.g.availability of sulfhydryl groups, content of native protein, hydration, particle size) as well as to final product quality (texture and sensory quality) and microstructure (characterized by confocal laser scanning microscopy).

What we know:

Pilot scale processing for dairy production (heat treatment, homogenization, acidification), basic measurements for aggregated milk protein properties (sulfhydryl groups, SDS-PAGE, particle size, HPLC), sensory test, CLSM, rheology and textural measurement.

What we need:

Techniques of surface plasmon resonance (SPR), isothermal titration calorimetry (ITC) and quartz crystal microbalance (QCM), and how to use them for characterizing and quantifying interactions between milk protein aggregates.

NO 2

Pathogenic Properties of Alzheimer's β-Amyloid Identified from Structure-Property Patient-Phenotype Correlations

Manish K. Tiwari[1,2] and Kasper P. Kepp[1,*]

[1]Department of Chemistry, [2]Department of Systems Biology, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

Ph.: +45 45 25 24 09 / E-mail: kpj@kemi.dtu.dk

β-amyloid (Aβ) plays a central role in Alzheimer's disease (AD), but the specific molecular mechanism and associated structures remain unknown. We compiled patient data for carriers of genetic variants of Aβ that cause AD and correlated these data against chemical properties for 56 mutant conformations derived from four published experimental conformations of Aβ of variable structure and chemical environment. Disease onset of variants is significantly ($p \sim 0.006$) correlated to hydrophobic surfaces of disordered conformations (2LFM), whereas structured conformations yielded no correlations. Correlation also applied ($p < 0.03$) to in vitro steady-state Aβ levels. We conclude that disordered monomers are likely to be pathogenically important in contrast to structured conformations and that hydrophobic surface correlates with pathogenesis. This first established correlation between clinical and chemical data suggests that specific exposed, disordered monomers are viable targets for AD therapy.

NO 3

Title: HEXPIN: Hetero-exopolysaccharide – milk protein interactions

Johnny Birch[1], Hörður Kári Harðarson[1], Sanaullah Khan[5], Richard Ipsen[2], Marie-Rose Van Calsteren[3], Christel Garrigues[4], Kristoffer Almdal[5], Maher Abou Hachem[1], Birte Svensson[1]

[1]Enzyme and Protein Chemistry, Dept. of Systems Biology, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark. [2]Dept. of Food Science, University of Copenhagen, DK-1958 Frederiksberg C, Denmark. [3]Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, Saint-Hyacinthe, Quebec J2S 8E3, Canada. [4]CED-Discovery, Chr Hansen A/S, DK-2970 Hørsholm, Denmark. [5]Dept. of Micro- and Nanotechnology, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

Today's food industry often uses restricted chemically modified polysaccharides (PS) in dairy products as thickeners and stabilizers. Hetero-exopolysaccharides (HePS) excreted by various lactic acid bacteria (LAB) strains are *generally recognized as safe* (GRAS) and have a similar positive impact on the textural properties in fermented milk products [1]. The textural effect stems in part from associative complexation between HePS and milk proteins, both caseins and whey proteins which complex with the casein micelle in milk during thermal processing. However, the molecular basis for the HePS-protein interactions and impact on textural properties is poorly understood and there is demand for understanding the HePS-protein interaction in order to be able to naturally increase viscosity in the final consumer product.

We produced and purified a series of HePS of known structures in yields of 41–135 mg l^{-1} from different *Streptococcus thermophilus* strains and 8–10 mg l^{-1} from *Lactobacillus rhamnosus* GG grown in skimmed milk medium. By using surface plasmon resonance (SPR), binding of the HePS to the whey proteins □-lactoglobulin or *α*-lactalbumin was monitored at different pH, ionic strength and temperature. HePS from *Lactobacillus rhamnosus* GG and *Streptococcus thermophilus* Sfi6 demonstrated an optimum in the binding capacity around pH 4.0 and 70 mM NaCl and decreasing binding capacity with increasing temperature in the range 20–35°C. Interaction at pH 4.0 between HePS and native or heat denatured □-lactoglobulin was supported by retardation in affinity gel electrophoresis. As a tool for exploring the potential of the structural variations represented by HePS, we established a database of structural and biophysical properties based on information on LAB HePS in the literature. HePS oligosaccharide repeat units will be prepared to allow accurate studies of the protein-carbohydrate interactions.

What we know:

- Homo-exopolysaccharides (HoPS) composed of various linear and branched a-glucans has no significant impact on formation of the textural matrix [2].
- HePS, which are composed of different monosaccharides (galactose, glucose, rhamnose, N-acetyl-galactosamine) contribute to texture and quality of yoghurt and cheese.
- Interactions between HoPS (□-glucans) and milk proteins (β-lactoglobulin and κ-casein) are specific and depend on linkage type, degree of branching and molecular weight of the HoPS.

- Eight purified HEPS of varying structure are available and more will produced also in larger amounts
- The HEPS show characteristic specificity in interaction with selected milk proteins.

What we need:

- To produce more HePS of known structure of LAB strains in larger amounts of high purity
- To identify smart ways of producing HePS oligosaccharide repeats
- To outline the rheological properties of the HEPS protein interacting systems
- To identify oligosaccharide repeats suitable for structure determination of protein complexes
- To consider hydrogen-deuterium exchange (HDX) as a method for identifying binding sites on milk proteins including heat-denatured proteins interacting with HEPS
- To develop other methods that connect structures of HEPS with functional properties

Acknowledgements
HEXPIN activities are supported by the Danish Research Council for Independent Research | Technical and Production Sciences and | Natural Science and a joint PhD stipend from DTU (to JB).

Literature

1 Badel, S., Bernardi, T. and Michaud, P. (2011). Biotechnol Adv, Elsevier Inc. **29**, 54–66.

2 Diemer, S. K., Svensson, B., Babol, L. N., Cockburn, D., Grijpstra, P., Dijkhuizen, L., Folkenberg, D. M., Garrigues, C. and Ipsen, R. H. (2012). Food Biophys **7**, 220–226.

NO 4

Investigating Antivenom Immune-profiling by high density peptide microarray

Mikael Engmark[13], Mikael Rørdam[1], Federico De Masi[2], Ole Lund[3].

[1]DTU Systems Biology, Network Engineering of Eukaryotic Cell Factories Group. [2]DTU Systems Biology, Regulatory Genomics Group. [3]DTU Systems Biology, Protein and Immune Systems Biology Group

Brief description of research area:

Venomous snakebites are regarded as one of the World's most neglected tropical conditions with up to 2.5 million victims every year[1]. The best-practice treatment is traditional antivenom derived from the blood of large mammals (typically horses or sheep) immunized with venom of one or more snake species. The active toxin-neutralizing components in antivenom are complex mixtures of antibodies (or fragments hereof)[2]. The individual antibodies are adapted by the immune system of the production animal to bind specific to parts of each toxin used in the immunization procedure. In many cases antivenom is also able to neutralize some – or even all – toxic effects of snakebites from related snake species[3]. Proteomics-based studies aiming at quantifying the extent of such cross-protection of antivenoms against venoms from related snake species are referred to as antivenomics. The current state-of-the-art antivenomics protocol involves affinity chromatography of venoms with immobilized antibodies[4]. Although proven effective in clinical applications antivenomics fail to explain how this cross-reactivity is working at the molecular level and must be performed for one snake venom-antivenom pair at a time.

Knowledge of interactions between the immunoreactive parts (referred to as epitopes) of a toxin or macromolecule in general and the corresponding antibodies is a prerequisite to understand and predict neutralization potential of a given antivenom against any fully characterized snake venom. Although antivenom to snakebites is a more than 120 years old invention[1], only little is known about the neutralizing antibodies or their epitopes[5].

This study aims at identifying snake-toxin-epitopes and characterizing important antibody-toxin interactions. This knowledge can be used to investigate conserved intra- and inter-species antibody binding sites and thereby potentially predict para-specificity of an antivenom. Such information can be used to guide future antivenomics experiments aiming at expanding the clinical applications of existing antivenoms to other snake species or suggest changes in immunization mixture to obtain an even better medicine. Furthermore, learning from nature's preferences for specific epitopes, it will be possible to conduct conservative estimations of the number of different neutralizing antibodies needed to neutralize the critical toxins from any characterized snake venom. This may in the long run result in recombinant immunization mixtures and even lead to the first fully recombinant antivenom.

What we know:

This study is based upon custom designed high-density peptide microarrays. Peptide microarray is a high-throughput and well-established technology used to study interactions between proteins and enable the identification of key residues in protein binding[6]. The technology works by synthesizing a large collection of different target peptides on a glass slide and allow antibodies from sera or other sources to bind to these. After several wasting steps to remove non-binding antibodies, a fluorescence-labeled secondary antibody is added and the glass slide is analyzed in a fluorescence scanner. Interactions between antibody sera and up to 2.9M different peptides can be studied on each slide. The microarrays in this study have been designed to contain 93'261 15-mer peptides derived 966 toxins from pit viper snake species (sub-family *Viperidae*). We are currently investigating the data and have identified a large number of linear epitopes.

What we need:

We need to investigate each epitope further to identify how many individual antibodies account for the observed tolerance in amino acid substitution. This implies additional peptide microarrays synthesis, protein modeling, and proteomics techniques combined with affinity purification of antibodies.

NO 5

Title: Detecting Steric Hindrances and Topological Changes of Proteins during Affine Morphs

Peter Røgen
Department of Applied Mathematics and Computer Science, Technical University of Denmark

Brief description of research area:
Structural comparison of protein structures are typically done using structural alignment programs such as RMSD, CE, Structural, GDT_TS, TM, Flex E, Dali, ……..
Each of these programs uses an underlying notion of distance that is blind to topological changes of the protein. I have therefor developed a program that can detect and score topological changes of a morph between two aligned protein structures.
What we know: Many homological proteins structures are e.g. despite low RMSD not topological similar and often protein structures classified as different folds are easy to morph into each other.
What we need: Collaboration with sequence alignment experts to investigate how this new and more detailed view on protein structure space may influence the view on protein sequence space.

Title: Protein structure refinement by optimization

Martin Carlsen1, Peter Røgen1
1Department of Applied Mathematics and Computer Science, Technical University of Denmark

Brief description of research area:
Application of mathematical methods such as linear algebra, convex optimization, b-splines and metrics for protein structure prediction. We are primarily concerned with the modeling of protein energy landscapes using a knowledge-based potential. Knowledge-based potentials are trained with the purpose of improving the quality of near-native protein structures. This means either to rank a set of near-native proteins according to how native-like they are (model quality assessment) or to refine the quality of near-native protein structures (protein structure refinement).
What we know: We have developed a smooth knowledge-based potential for protein structure refinement using a modified Newton method as our refinement method and an iterative method to improve the performance of our potential. Using this strategy we were able to improve the quality of many near-native structures.
What we need: Our potential is trained on the Titan-HRD set generated by torsion angle dynamics which consists of non-homologous proteins. We would like to train our potential on a homologous training set instead. Our potential may also serve as components in a molecular dynamics potential.

NO 6

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What we need: Our potential is trained on the Titan-HRD set generated by torsion angle dynamics which consists of non-homologous proteins. We would like to train our potential on a homologous training set instead. Our potential may also serve as components in a molecular dynamics potential.

NO 7

The *Lactococcus lactis* Thioredoxin Reductase - a closer look

Nicklas Skjoldager[1], Olof Björnberg[2], Maria Blanner Bang[3], Pernille Harris[3], Birte Svensson[2], Per Hägglund[1].

[1]*Protein and Immune Systems Biology, DTU Systems Biology.*

[2]*Enzyme and Protein Chemistry, DTU Systems Biology.*

[3] *DTU Chemistry*

Brief description of research area:

Lactococcus lactis is an industrially important Gram-positive, non-sporulating microaerophilic bacterium which is commonly used as a starter culture in production of cheese, butter milk and wine. *L. lactis* is equipped with a thioredoxin disulfide reduction system composed of a NADPH-dependent thioredoxin reductase (TrxR) and two thioredoxins (TrxA and TrxD), which in turn reduce disulfide bonds in target proteins: NADPH --> TrxR --> TrxA/TrxD --> target proteins --> reductive cellular processes.

TrxR is a flavoenzyme and relies on the tightly bound co-factor FAD, in order to receive the reducing equivalent from NADPH. Reduction of FAD is followed by a change in-between the two conformations named FR and FO. Similar to most other lactic acid bacteria *L. lactis* does not have glutaredoxin and the enzymes to synthesize glutathione. Therefore *L. lactis* relies exclusively on the thioredoxin system in order to keep the cytosol in the proper reduced state. Furthermore *L. lactis* is catalase-negative and thus likely to rely on thioredoxin-dependent thiol peroxidases to remove hydrogen peroxide.

What we know:

Recent studies have shown that the TrxR from *L. lactis* can reduce molecular oxygen by consumption of NADPH. Surprisingly, the TrxR is inactivated by visible light, a feature not observed in the homologous enzymes from *Escherichia coli* and barley (*Hordeum vulgare*). Spectrophotometric analysis suggests that the loss of activity is related to a modification of the enzyme-bound FAD. Mass spectrometry of the FAD reveals a mass gain of 13.98 Da, corresponding to the formation of an aldehyde[1]. To get further insight into these unique features of *L. lactis* TrxR, several three-dimensional structures of native and light-inactivated forms have been solved at high resolution by X-ray crystallography, both in FO and FR conformations.

What we need:

We want to understand the mechanism behind the modification of FAD

What is the precise modification(s) of the FAD?

Why does the modification of FAD lead to loss in activity?

What is the special feature of *L. lactis* TrxR that causes it to be light sensitive?

1. Björnberg, O. *et al.* Lactococcus lactis Thioredoxin Reductase Is Sensitive to Light Inactivation. *Biochemistry* 150220134459000 (2015). doi:10.1021/bi5013639

NO 8

Title: Differential Proteomics and Adhesion Analyses of Interactions between *Lactobacillus acidophilus* NCFM, oligosaccharides, Mucin, and Dietary Phenolics

Hasan Ufuk Celebioglu[1], Sampo Lahtinen[2], Susanne Brix[1], Maher Abou Hachem[1], Susanne Jacobsen[1], and Birte Svensson[1] [1]*Department of Systems Biology, DTU.* [2]*DuPont, Health & Nutrition*.

Brief description of research area:

Lactobacillus acidophilus NCFM (NCFM) is one of the well-defined probiotics that was isolated from a human source and since then has been used intensively in foods and dietary supplements, as well found as commercially. Its adhesion was studied previously and the proteins that are responsible for the attachment of the bacterium to intestinal cells and mucus layer were identified. Prebiotics are non-digestible food constituents that beneficially affect the host health by stimulating the selective growth of commensal bacteria in the gastrointestinal tract. There is a number of emerging prebiotics including fructooligosaccharides (FOS), cellobiose, melibiose, isomaltulose. Although the metabolism mechanisms of the carbohydrate utilization have been studied commonly, there are few or no studies about how these carbohydrates can impact on the functional probiotic properties of these bacteria. The gut microbiota is able to modulate the production, bioavailability and, the biological activities of dietary phenolics. Also dietary phenolics can modulate the composition of gut microbiota.

This study aimed to investigate the interactions between probiotic bacterium *Lactobacillus acidophilus* NCFM, defined and emerging prebiotic oligosaccharides as carbon source, the protein component of the mucus layer, mucin, and dietary phenolics by adhesion and proteome analyses.

What we know:

We have preliminary results for adhesion of NCFM grown on different oligosaccharides as carbon source, or mucin, or different dietary phenolics, such as resveratrol and tannic acid. We showed some of oligosaccharide utilization changed the adhesion as well as surface proteins which could have roles in adhesion. In addition, we also identified “moonlighting” proteins on the surface of the bacterium whose abundancy were changed. Our results indicate that the high adhesiveness of the bacteria is dependent on carbon source or environmental change; and increased adhesion is favorable to have positive impacts on host health.

What we need:

We will continue to analyze the effects of dietary phenolics on proteome profile. In addition, we need to identify if NCFM metabolize these selected phenolics and what are the metabolic products.

NO 9

Discovery of Peptide-based Inhibitors against Dendrotoxin B from Black Mamba through Phage Display Screening

SaioaOscoz Cob1, Andreas Munk1, Andreas Hougaard Laustsen2, and Brian Lohse2
1Department of Systems Biology, Technical University of Denmark
2Department of Drug Design and Pharmacology, University of Copenhagen

Brief description of research area:

Snakebite envenomation is an underestimated and neglected public health issue with more than 5.5 million cases per year, resulting in 125,000 deaths. Antivenoms are still being produced using traditional animal immunization protocols, which are associated with safety issues,including death, due to their high immunogenicity in humans. Consequently, a novel approach is needed to produce safer, more efficacious, and cheaper antivenoms. The goal of this researchis to develop inhibitors against the medically most relevant toxins in snake venoms, in order to make a polyvalentantivenom consisting of monoclonal antibodies and/or peptides, paving the way for a fully recombinant or synthetically produced antivenom.
The first step has been to focus ondendrotoxin B (Dtx B), which belongs tothe Kunitz- type proteinase inhibitor family.These are the most abundant toxins in black mamba (*Dendroaspispolylepis*). Dendrotoxinsare poorly neutralized by current antivenoms, and as they are almost impossible to raise an immune response against. Here we report the discovery of peptide-based antitoxins against Dtx Bthrough phage display screening. The DNA from several phages that bound strongly to thedendrotoxiniscurrently being sequenced and later studies will reveal if the corresponding peptides are able to inhibit the toxic effects of dendrotoxins.
If strongly binding peptides are identified, we are looking for collaborators who are interested in grafting the peptides onto a human antibody scaffold in order to create a biopharmaceutical with extended half-life that can be expressed by CHO cell fermentation.

NO 10

Structure of the DBL4γ Domain of a Malaria Parasite Erythrocyte Membrane Protein 1 and Identification of the Binding Site for a DBL4γ-specific Monoclonal Antibody.

Vladislav Soroka[a,b], Lea Barfod [a], Harm Otten[b], Kasper Rand [c], Anja Bengtsson[a], Anja T.R. Jensen[a], and Sine Larsen[b]

[a] *Center for Medical Parasitology, University of Copenhagen and Copenhagen University Hospital (Rigshospitalet).*

[b] *Department of Chemistry, University of Copenhagen.*

[c] *Department of Pharmacy, University of Copenhagen.*

Malaria remains a serious health problem affecting 198 million people worldwide each year. In 2013 alone, WHO reported 584.000 deaths caused by malaria parasites. The main source of fatal complications is the ability of infected erythrocytes (IE) to adhere to host endothelium cells in brain or placenta capillaries where they obstruct blood circulation and cause inflammation. Adhesion of IE is mediated by the parasite *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1). PfEMP1 is a large group of multimodular adhesion receptors consisting of a N-terminal segment, a variable number of Duffy-binding-like (DBL) domains together with one or two Cysteine-rich Inter-domain Region (CIDR) domains. DBL domains are further divided into seven subclasses (α, β, γ,δ, ε, ξ, x) based on sequence similarity.

We present the structure of PFD1235w DBLγ, a 400 residues long PfEMP1 domain, which is known to bind a specific antibody (AB01) generated from B-cells isolated from a *P. falciparum*-infected individual living in Ghana. To our knowledge, this is the first known structure of a DBLγ subclass domain.

DBL4γ had proven to be a difficult target that presented several major problems starting from purification and crystallization to diffraction data analysis and structural determination. Despite the presence of 20 cysteines and 9 disulfide bonds, we managed to express DBL4γ in *E. coli* as correctly folded protein. The initial structure was determined by SeMet MAD phasing at 3.5Å and

followed by SAD phasing using Hg derivative at 2.8Å. Finally, the 2.0Å structure of PfEMP1 DBLγ domain was refined using native data collected at the ESRF microfocus beamline. The boomerang shaped α-helical core of the protein domain consisting of three subdomains is similar to the previously determined DBL domains, but the conformation of the extended loops is very different. Using hydrogen-deuterium exchange mass spectroscopy (HDX-MS), we identified the AB01 binding site to one of the extended loops in the N-terminal subdomain of DBL4γ.

NO 11

Title: Isolation and Characterization of novel algae degrading enzymes from an agar-degrading marine *γ*-proteobacterium.

Pernille Bech, University of Copenhagen Section for Genetics and Microbiology Thorvaldsesvej 40, 2nd floor DK-1871 Frederiksberg C Copenhagen, Denmark

A marine *γ*-proteobacterium was recently isolated from seawrack due to its conspicuous burial in solid agar. Genome sequencing revealed a great potential for degradation of algal polysaccharides, where 79 putative glycosyl hydrolases (GHs) were annotated, of which six putative agarases, a *κ*-carrageenase, a *ι*-carrageenase and a neoagarobiose hydrolase were identified from a protein blast. A phylogenetic analysis of the nine GHs showed that they all were distant related to their respective GH families i.e. less than 40% identity on protein level. One agarase together with a *κ*-carrageenase has successfully been expressed in *Escherichia coli* with substrate specificity on agar and *κ*-carrageenan, respectively. The two enzymes were expressed as a fusion protein with a His-tag to its C-terminal and purified on HiTrap HP column.

A functional expression library was made as another approach to access un-annotated glycosyl hydrolases from the bacterium. Novel ekstracellular enzyme activities were identified. Two *α*-amylases together with a mannanase and cellulase were found.

NO 12

Investigation of the Substrate Specificity Determinants of Barley Limit Dextrinase

Susan Andersen[1], Marie S. Møller[2], Maher Abou Hachem[1] & Birte Svensson[1]

[1]*Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, 2800, Kgs. Lyngby, Denmark; e-mail: sjes@bio.dtu.dk.*

[2]*Department of Biochemistry and Structural Biology, Department of Chemistry, University of Lund 221 00 Lund, Sweden.*

Brief description of research area:

The barley limit dextrinase (LD), a type I pullulanase (EC 3.2.1.41), is the only debranching enzyme able to hydrolyse α-1,6-glucosidic linkages in branched limit dextrins present in the germinating seed (Kristensen et al., 1999), and therefore considered important during malting and brewing. In addition, LD is also capable of hydrolysing α-1,6 linkages in pullulan and, with less efficiency, 1,6-branching points in amylopectin (Kristensen et al., 1999; Burton et al., 1999).

Recently published three dimensional crystallographic structures of LD and structural comparisons with related α-1,6-acting and α-1,4-acting glycoside hydrolase family 13 enzymes suggests several substrate specificity determinants of LD (Møller et al., 2015). In addition, the variation in enzymatic activity has also been suggested to stem from mutations in two amino acid residues located in the N-terminal domain of *Sorghum bicolor* LD (Gilding et al., 2013). The function of the abovementioned amino acid residues is to be experimentally determined by mutational analysis.

What we know:

Recombinant protein production in *Pichia Pastoris*, protein purification, mutational analysis, protein-carbohydrate interactions and substrate specificity determination.

What we need:

Branched maltooligosaccharides; containing α-(1-4) glycosidic linkages and α-(1-6) linkages at branching points.

Kristensen et al., Biochim. Biophys. Acta. Protein. Struct. Mol. Enzymol. 1431, 538-546 (1999)
Burton et al., Plant Physiol. 119, 859-871 (1999)
Gilding et al., Nat. Commun. 4, 1483 (2013)
Møller et al., J. Mol. Biol. 427, 1263–1277 (2015)
Stam et al., Protein Eng. Des. Sel. 19, 555–562 (2006)

NO 13

Title: Protein Carbonylation in oxidative stress and disease.

Martin Rykær[1,2,3], Birte Svensson[2], Kim Henriksen[3], Per Hägglund[1].

[1]*Protein and Immune Systems Biology, DTU Systems Biology*

[2]*Enzyme and Protein Chemistry, DTU Systems Biology*

[3]*Nordic Bioscience A/S.*

Brief description of research area:

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

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

What we know:

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

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

What we need:

Method development and optimization; purification of and enrichment for, carbonylated peptides and proteins.

NO 14

Title: How food structure affects satiety - StrucSat

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Brief description of research area:

Obesity is an increasing problem for modern society. Food for weight management is designed with lower energy content as compared to their conventional counterparts. Alternatively food could be designed with similar energy content (isoenergic) but different effects on satiety. The goal of the project is to elucidate how food structure affects satiety by designing well characterized foods consisting of whey proteins and alginate. Alginate is an indigestible polysaccharide from brown algae consisting of (1→4)-β-D-mannuronic acid and α-L-guluronic acid. The PhD project at DTU aims to characterize the interaction between native as well as cross-linked whey proteins and alginate by ITC, DSC and DLS and how their structure affects their simulated gastric breakdown assessed by mass spectrometry.

What we know:

Alginate and β-lactoglobulin (βLg) interacts below the pI of the protein, but above the pI of alginate and form insoluble complexes with a Kd of approximately 4 μM. βLg and alginate dissociates if the pH is increased beyond the pI of βLg and fluorescence scanning experiments performed on the dissociated βLg indicate that if there is structural change when the complexes precipitate it is reversible. Furthermore, the ζ-potential of alginate of different molecular weight and βLg has been assessed.

What we need:

-FTIR spectroscopy to asses structural change in insoluble complexes.

-Quartz crystal microbalance access for studying insoluble complex formation

-Well-defined alginate oligosaccharides for model protein interaction studies.

NO 15

Title: Decoding Nitrosylation in Bacteria and Humans

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Brief description of research area:

Many cellular functions and pathways are regulated via posttranslational modifications of proteins. These protein modifications can result in a variety of outcomes including changing the activity, function and location of the protein and hence cause critical perturbations to the cellular state. Modification of protein cysteine residues with nitric oxide (NO) is one of the modifications that in recent years have gained a lot of attention. By a process called S-nitrosylation the nitric oxide reacts with the thiol group of a cysteine residue, resulting in the formation of a nitroso group on the cysteine.

It has become evident that failure in the regulation of protein S-nitrosylation is the cause of a broad range of diseases including inflammatory diseases such as fibrotic pulmonary disorders and rheumatoid arthritis, neurodegenerative disorders and cancer.

The overall aim of this PhD study is to use mass spectrometry-based proteomics to identify and characterise S-nitrosylated proteins and peptides and hereby:

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

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

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

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

NO 16

Title: "Mass spectrometry-based structural characterization of crosslinked Peptides"

Michele Mariotti

Brief description of research area:

The main goal of this project is to investigate the structure of cross-linked proteins and peptides in various biological samples with focus on the extracellular matrix, using MALDI-TOF and LC-MS/MS techniques. These are also promising techniques in the identification of biomarkers related to different diseases correlated to cross-linking, such as pulmonary fibrosis and kidney diseases. Cross-links that I will investigate in my research include for example enzymatic cross-links (catalyzed by transglutaminase, lysyl oxidase and other enzymes), non-enzymatic cross-links formed spontaneously, cross-links generated by a photochemical process and chemically induced cross-links.

What we know:

I am currently using mass spectrometry techniques such as MALDI-TOF and LC/MS-MS, and I can use them to investigate the structures of different digested protein samples. ^{18}O isotope labeling is a useful technique that I am applying to obtain better results in my study. I am currently gaining expertise in different proteomics software, with focus on MaxQuant, and recent software for detection of post-translational modifications and cross-linking, such as Proteios and MassAI.

What we need:

The challenge of this project is to develop an efficient strategy in order to discover and identify cross-links in different biological samples that are potentially cross-linked, without knowing the chemistry of the cross-links in advance. I would also need to use novel proteomics tools and programs to analyze my results from mass spectrometry with the intent of identifying cross-links.

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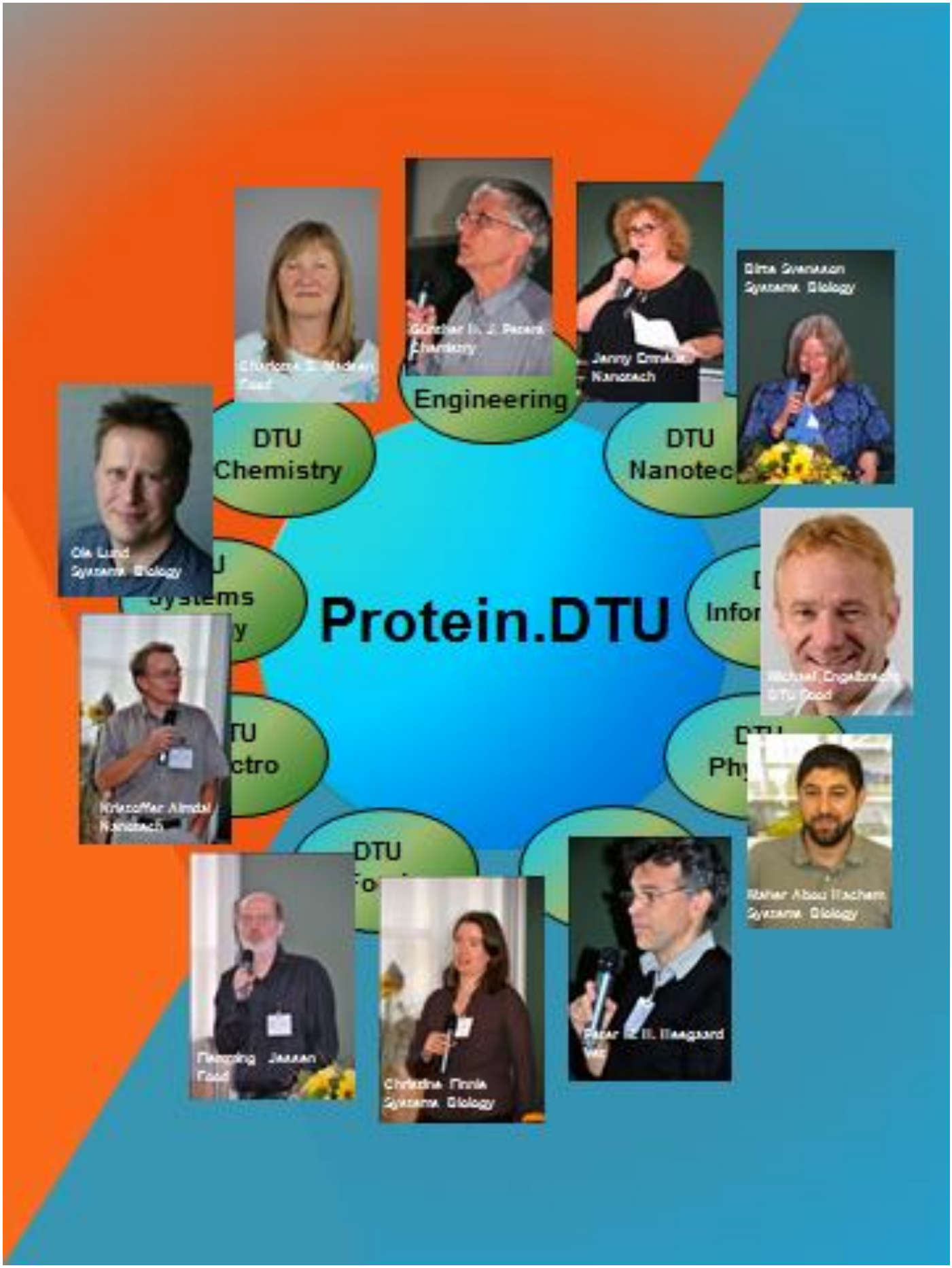
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