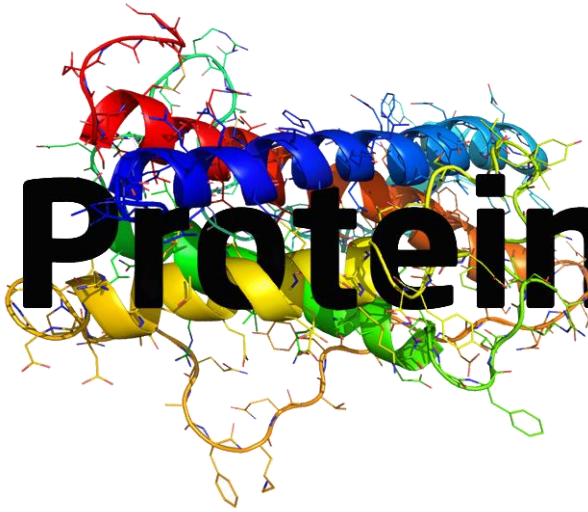
Applied Protein Science

14th Protein.DTU Workshop



May 9, 2016

Applied Protein Science
14th Protein.DTU Workshop

Contents

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May 9, 2016
Building 208, Lecture Hall 51 – Kemitorvet, 2800 Kgs. Lyngby

Program

08:30 – 09:00 **Arrival & Breakfast**

09:00 – 09:10 **Welcome**
Martin P. Bendsøe (Senior Vice President and Dean, DTU)
Chair: Birte Svensson (Professor, DTU Systems Biology)

09:10 – 09:40 **Polysaccharide Breaking News. Lytic polysaccharide monooxygenases: potential, structures and substrate binding**
Leila Lo Leggio (Associate Professor, KU Chemistry)
Chair: Maher Abou Hachem (Associate Professor, DTU Systems Biology)

09:40 – 10:10 **Recombinant human albumin: Mechanisms behind its use as a versatile stabiliser for hard-to-formulate biopharmaceuticals**
Line Abildgaard Ryberg (PhD student, DTU Chemistry)
Chair: Günther Peters (Associate Professor, DTU Chemistry)

10:10 – 10:40 **Advanced detection of immune reactivity using assembled protein complexes presenting personalized epitopes**
Sine Reker Hadrup (Associate Professor, DTU Vet)
Chair: Peter Heegaard (Professor, DTU Vet)

10:40 – 11:10 **Coffee Break**

11:10 – 12:20 **FLASH PRESENTATIONS - 4 min each**
Chairs: Kristoffer Almdal (Professor, DTU Nanotech)
Charlotte B. Madsen (Senior Scientist, DTU Food)
Pernille Harris (Associate Professor, DTU Chemistry)

12:20 – 13:30 **Lunch and poster viewing**

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Program - *continued*

13:30 – 14 00 **Ultra sensitive detection of Alzheimer disease biomarkers**
Martin Dufva (Associate Professor, DTU Nanotech)
Chair: Flemming Jessen (Senior Scientist, DTU Food)

14:00 – 14:30 **What can high pressure do for protein structure and functionality in food?**
Vibeke Orlien (Associate Professor, KU, Faculty of Science, Ingredient and Dairy Technology)
Chair: Flemming Jessen (Senior Scientist, DTU Food)

14:30 – 15:00 **The structure of dopamine β-hydroxylase: The enzyme that converts dopamine to noradrenaline**
Hans Erik Mølager Christensen (Associate Professor, DTU Chemistry)
Chair: Pernille Harris (Associate Professor, DTU Chemistry)

15:00 – 16:30 **Poster session, coffee and "time for a beer"**
Chairs: Maher Abou Hachem (Associate Professor, DTU Systems Biology)
Flemming Jessen (Senior Scientist, DTU Food)
Peter Heegaard (Professor, DTU Vet)

List of Poster Abstracts

Abstract no

21 Saioa Oscoz1: Development of peptidic anti-dendrotoxins
22 Sanaullah Khan: Solution scattering studies of bacterial exopolysaccharides and their interactions with β-lactoglobulin
23 Sita Vaag Olesen: Characterisation of non-histone N-ε acetylation in probiotics
24 Susan Andersen: Investigation of the Substrate Specificity Determinants of Barley Limit Dextrinase
25 Triinu Visnapuu: Transglycosylation reaction of bacterial *N*-acetyl hexosaminidases towards human milk oligosaccharide core structures
26 Yong Wang: Experiment Driven Simulations Provide a Global View on the Native-state Free Energy Landscape of a T4 Lysozyme Mutant
27 Yuya Kumagai: Preparation of linear isomaltomegalosaccharide by *Gluconobacter oxydans* dextran dextrinase

NAD^+ and NADH effect on sirtuin deacetylation - an *in silico* study

Ana Rita Colaço[1], C. A. Olsen[1,2] and P. Fristrup[1]
[1]Technical University of Denmark, Department of Chemistry, 2800 Kgs. Lyngby, Denmark. [2]University of Copenhagen, Faculty of Health and Medical Sciences, Center for Biopharmaceuticals, 2100 Copenhagen, Denmark

Brief description of research area
The field of epigenetics has received considerable attention in recent years due to its impact on genetics, developmental biology, cancer biology, and medicinal chemistry. This research project will focus on a family of epigenetic regulator enzymes, with homology to the silent information regulator 2 yeast enzymes, known as sirtuins. This is a family of NAD^+-dependent enzymes that catalyze the deacetylation of histone and non-histone proteins. There are seven proteins in the sirtuin family and they all share a conserved 270 amino acid catalytic domain, with variable N- and C- termini. This class of enzymes is implicated in several cellular processes, and have been suggested as therapeutic targets for diabetes, cancer, neurodegenerative diseases and inflammation.

What we know
We hypothesize that sirtuins may differentiate between substrates through recognition of peptide sequence (which has been well established in the field), as well as through recognition of acyl group identity. The latter concept was originally based on structural and mechanistic data reported for inhibitors of these enzymes, and qualitative scrutiny of the growing number of available crystal structures. However, recent publications appearing in the journals Cell, Science, and Nature have shown that acyl-lysine modifications other than acetyl are indeed biologically relevant, which have substantiated our main hypothesis considerably.

Several in vivo studies have described NADH as a weak competitive inhibitor for yeast sirtuins, however this has yet to be tested for mammalian sirtuins. As a starting point in this in silico study, we investigate the NADH inhibition of SIRT1, SIRT3 and SIRT5 using long Molecular Dynamics (MD) simulations and Density Functional Theory (DFT) calculations. Our results showed that the weak inhibition effect of NADH is highly correlated with the misalignment of the nicotinamide amide to the key residues ILE (interleucine) and ASP (aspartate) in the C pocket of the sirtuin.

What we need
The increased complexity of the field has emphasized the need for novel tools to investigate the substrate specificity of sirtuins. We plan to use state-of-the-art computational methods, chemical synthesis techniques, as well as biochemical profiling to design and evaluate novel substrate candidates. The computational study will be initiated with homology modelling of SIRT4 and SIRT7. These homology models will be subjected to long molecular dynamics (MD) simulations both in complex with known inhibitors and substrates as well as alone. The determined X-ray crystal structures will also be included in the MD study in order to enable characterization of the relevance of the solid-state structures with regard to the structures in solution. The structures obtained from our MD simulations will be validated by a comprehensive docking study using sirtuin inhibitors reported in the literature. A mixed quantum mechanical/molecular mechanics (QM/MM) study of the reaction pathways for a series of acylated lysine substrates will be performed in order to recognize particularly favorable enzyme–substrate combinations, which may have physiological relevance. Based on the collective QSAR information obtained from the modelling studies, we will design, synthesize, and screen a focused collection of short peptides.

Mass spectrometry analysis of hydrolytic action and transglycosylation by β-mannanases

Anna Rosengren[1], Johan Morrill, Sumitha Reddy and Henrik Stålbrand

Department of Biochemistry and Structural Biology, Lund University, PO Box 124, SE-221 00, Sweden

Brief description of research area

Hemicellulose such as *O*-acetyl galactoglucomannan (GGM) is a renewable resource for novel oligomeric and polymeric products [1]. Hydrolysis of the GGM backbone is catalysed by β-mannanases. These enzymes use a retaining double displacement mechanism and can in principle carry out transglycosylation, i.e. enzymatic synthesis of β-mannosidic bonds – arguably the most difficult glycosidic bond to synthesise chemically. Structural and biochemical characterization of homologous β-mannanases have revealed that, although sharing the same overall fold and catalytic mechanism, there are differences in their hydrolytic action, ability to perform transglycosylation and also in acceptor specificity [3-7].

What we know

To study subsite interactions and map the binding of oligosaccharides in the active site we have developed a method where solvent isotope labeling using $H_2{}^{18}O$ is combined with mass spectrometry and HPAEC-PAD analysis [2-3]. We also use mass spectrometry (MALDI-TOF and Orbitrap) for analysis of acceptor specificity in transglycosylation reactions.

What we need

The capacity of β-mannanases to synthesize alkyl-glycosides (surfactants) from saccharides and alcohols is of particular interest. Alkyl-glycosides are mild and biodegradable surfactants with potential use in cleaning and hygiene products as well as in pharmaceutical formulations. We work on scaling up the enzymatic reactions for further purification and characterization of alkyl-glycosides, using reversed phase chromatography and NMR.

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Isoenergically limiting digestibility of whey protein by crosslinking with transglutaminase

Emil G. P. Stender[1], Glykeria Koutina[3], Richard Ipsen[3], Kristoffer Almdal[2], Birte Svensson[1]
[1]Technical University of Denmark – DTU – Department of Systems Biology. [2]Technical University of Denmark – DTU – Department of Micro- and Nanotechnology. [3]University of Copenhagen – Department of Food Science.

Brief description of research area
Satiety is an inhibitory mechanism in humans and other mammals taking place post ingestion of food which prevents the return of hunger for a variable time period. (1) Satiety might be affected by nutrient status (1) and interluminal viscosity. (2) The uptake of amino acids is influenced by the digestibility of proteins. A protein which is in an extended conformation is digested more rapidly than a compact aggregate. (3) Transglutaminase is used to crosslink whey proteins to a large extent in order to isoenergically alter the structure of the proteins and consequently digestibility. The whey protein mixture primarily consists of β-lactoglobulin and α-lactalbumin.

What we know
Native β-lactoglobulin is resistant to crosslinking by transglutaminase, α-lactalbumin is not. When the whey protein is denatured either by heat or by increasing pH to more than pH 10 and returning pH to 6.3, β-lactoglobulin is permanently denatured and becomes crosslinkable. Heat denatured whey proteins form large insoluble aggregates due to cysteine crosslinking by disulfide bond formation. pH denatured whey proteins form soluble aggregates crosslinked by disulfide bond formation but to a lesser extent than heat denatured whey protein. When the denatured protein is crosslinked the particle hydrodynamic diameter as assessed by dynamic light scattering (DLS) is increased but the pH denatured crosslinked whey protein remains soluble. Both forms of denaturation increase the solvent exposed hydrophobic surface compared to native whey proteins, subsequent crosslinking decrease the solvent exposed hydrophobic surface as assessed by 8-Anilino-1-naphthalenesulfonic acid (ANS) fluorescence. The *in vitro* gastric and intestinal digestion of the proteins was investigated and followed by the release of trichloroacetic acid (TCA) soluble peptides. Crosslinking of whey proteins decreases the release of TCA soluble peptides in the gastric digestion phase as compared to the starting material. However the extended conformation of the pH denatured proteins increase the release of TCA soluble peptides during gastric digestion as compared to native whey protein even when the pH denatured protein is crosslinked. Heat treating whey protein as well as crosslinking heat treated whey protein decrease the release of TCA soluble peptides during gastric digestion. Only a minor decrease in intestinal digestibility of crosslinked whey protein was observed as compared to native whey protein.

What we need
Assessment of the morphology of the aggregates possibly through AFM, SEM or TEM.
Assessment of the solvent accessible surface of the particles.

Acknowledgements
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Structural characterization of the thermostable *Bradyrhizobium japonicum* D-sorbitol dehydrogenase

Folmer Fredslund[a*], Harm Otten[a,d*], Sabrina Gemperlein[b], Jens-Christian N. Poulsen[a], Yvonne Carius[c], Gert-Wieland Kohring[b] and Leila Lo Leggio[a]

[a]Department of Chemistry, University of Copenhagen, Universitetsparken 5, Copenhagen, DK-2100, Denmark; [b]Microbiology, Saarland University, Campus, Bldg. A1.5, Saarbrücken, Saarland, D-66123, Germany; [c]Department of Structural Biology, ZHMB, Saarland University, Building 60, D-66421 Homburg, Germany; [d]present address: Novozymes A/S, Kroghoejsvej 36, 2880 Bagsvaerd, Denmark; *These authors contributed equally

Brief description of research area

We work on enzymes and investigate their function and reaction mechanisms. Of particular interest are the structural determinants of substrate specificity in glycoside hydrolases, lytic polysaccharide monooxygenases (LPMO's) and other enzymes useful in an industrial setting, such as the presented D-sorbitol dehydrogenase, that can be used to produce D-sorbose with a very high enantioselectivity.

What we know

The group has several years of experience in structural investigations on proteins, using X-ray crystallography is the central technique, complemented by spectroscopy, small angle scattering and biochemical/protein chemistry techniques.

What we need

We are looking for collaboration partners to engage in common projects on industrial relevant enzymes for applied research, where we can provide the basic research.

Interactions of dietary phenolics with the probiotic bacterium *Lactobacillus acidophilus* NCFM revealed by adhesion assays and differential proteomics

Hasan Ufuk Celebioglu[1], Marta Delsoglio[1,2], Sampo Lahtinen[3], Susanne Brix[1], Maher Abou Hachem[1], and Birte Svensson[1]

[1]Department of Systems Biology, DTU. [2]Department of Life Sciences and Systems Biology, University of Turin. [3]DuPont, Health & Nutrition.

Brief description of research area

Dietary phenolics are secondary metabolites of plants abundantly found in fruits, vegetables, cereals and beverages where they can confer bitterness, astringency, color, flavor, odor and oxidative stability. *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. 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 and dietary phenolics (resveratrol, tannic acid, caffeic acid, and ferulic acid) by adhesion to mucin and human intestinal HT-29 cells, and whole-cell, as well as surface proteome analyses.

What we know

We have preliminary results for adhesion of NCFM treated with different concentrations of resveratrol and tannic acid. We showed the adhesion can be altered in concentration-dependent manner. Moreover, some proteins from whole-cell proteome are differentially abundant in phenolics-treated growths.

What we need

We will continue to analyze the effects of other dietary phenolics (caffeic and ferulic acids) on proteome profile (both whole-cell proteome and surface proteome) and adhesion (to mucin and human intestinal HT-29 cells) properties. In addition, we need to identify if NCFM metabolize these selected phenolics and what the metabolic products are.

Illuminating the binding interactions between the JJ1 inhibitor and α-cobratoxin by HDX-MS

Henrik Flindt,[1] Tam T. T. N. Nguyen[1], Andreas H. Laustsen[2], Brian Lohse[2], and Kasper Rand[1]

[1]Copenhaven University (SUND) at the School of pharmaceutical Sciences , Protein analysis Group
[2]Copenhaven University (SUND) at the School of pharmaceutical Sciences , Department of Drug Design and Pharmacology

Brief description of research area
A follow up on the work done by Mireia Solà "Optimization of anti-cobratoxins for treatment of neurotoxic envenomings" (Also presenting at this workshop)
Overall theme for this is the JJ-1 inhibitor of α-cobratoxin. Binding site study utilizing HDX, to help evaluate JJ-1 potential as a universal antidote.

What we know
A peptide JJ-1 inhibits α-cobratoxin but the binding arear is unknow

What we need
To localize the binding arear. If localized, the amino sequence in question will be used in a cross-comparisments between homologous toxins from other snake species to evaluate JJ-1 ability as a cross-reactive inhibitor.

HEXPIN: Hetero-exopolysaccharide – milk protein interactions

Johnny Birch[1], Hörður Kári Harðarson[1], Sanaullah Khan[2], Emil G. P. Stender[1], Birthe B. Kragelund[3], Marie-Rose Van Calsteren[4], Christel Garrigues[5], Maher Abou Hachem[1], Richard Ipsen[6], Kristoffer Almdal[2], Birte Svensson[1]

[1]Enzyme and Protein Chemistry, Dept. of Systems Biology, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark. [2]Dept. of Micro- and Nanotechnology, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark. [3]Dept.of Biology, University of Copenhagen, 2200 Cph-N, Denmark. [4]Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, Saint-Hyacinthe, Quebec J2S 8E3, Canada. [5]CED-Discovery, Chr Hansen A/S, 2970 Hørsholm, Denmark. [6]Dept. of Food Science, University of Copenhagen, 1958 Frederiksberg C, Denmark.

Brief description of research area

Hetero-exopolysaccharides (HePS) excreted by various lactic acid bacteria (LAB) strains are *generally recognized as safe* (GRAS) and have a positive impact on textural properties of fermented milk products [1]. The effect stems in part from associative complexation between HePS and milk proteins. However, the molecular basis for the HePS-protein is poorly understood and more insight is required to rationally design texture and viscosity properties in the final consumer product.

We produced and purified a series of HePS of known structures in yields of 70–350 mg l^{-1} from different *Streptococcus thermophilus* strains in the molecular weight range of 138±3–4683±442 kDa as determined by size exclusion chromatography (SEC). Binding of individual HePS to *β*-lactoglobulin, *β-casein, κ-casein,* and heat treated *β*-lactoglobulin was assessed at different pH, ionic strength and temperature using surface plasmon resonance (SPR). HePS–*β*-lactoglobulin showed highest binding capacity around pH 4.0 and 70 mM NaCl decreasing with increasing ionic strength and temperature (20–35°C). The optimum for HePS-native *β*-lactoglobulin complexation at pH 4.0 was supported by dynamic light scattering analysis. Oligosaccharide repeating unit produced by mild acid hydrolysis of HePS from *Streptococcus thermophilus* LY03 and anionic oligosaccharide from aliginate produced by aid of alginate lyase were prepared and their binding to *β*-lactoglobulin was analyzed by NMR spectroscopy at pH 2.65. Chemical shift perturbation analysis revealed two and one potential binding sites for the HePS and alginate oligosaccharides, respectively.

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.

What we know

- Homo-exopolysaccharides (HoPS) composed of various linear and branched α-glucans have no significant impact on formation of the textural matrix [2].
- HePS, which are composed of different monosaccharides (galactose, glucose, rhamnose, N-acetyl-galactosamine, N-acetyl-glucosamine) contribute to texture and quality of yoghurt and cheese.
- Interactions between HoPS (α-glucans) and milk proteins (β-lactoglobulin and κ-casein) depend on linkage type, degree of branching and molecular weight.
- Eight purified HePS of varying structure are available and for some repeating unit oligosaccharides are produced
- The HePS show characteristic specificity in binding capacity and affinity for selected milk proteins.

What we need

- To outline rheological properties of the HePS protein interacting systems
- To determine HePS oligosaccharide-protein complexes by x-ray crystallography
- To introduce hydrogen-deuterium exchange (HDX) as a method for identifying HePS binding sites on milk proteins including heat-denatured proteins
- To develop methods that connect structures of HePS with functional properties

Acknowledgements

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Immune repertoire profiling by high-throughput sequencing

Joseph John Kaplinsky[1], Ramy A. Aranout[2,3], Thomas Lars Andresen[1]

[1]Department of Micro- and Nanotechnology DTU Nanotech Technical University of Denmark Ørsteds Plads 345B, 2800 Kgs., Lyngby, Denmark. [2]Department of Pathology and Division of Clinical Informatics, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215. [3]Department of Systems Biology, Harvard Medical School, Boston, MA 02115

Brief description of research area

The adaptive immune system generates tremendous proteomic diversity in the antibody and T-cell receptor repertoires. This diversity can now be probed by high throughput sequencing which allows receptors in many thousands of cells per sample to be sequenced. The resulting data can inform insights from the systems level (e.g. co-evolution of pathogens with human immunity) down to the level of molecular binding (e.g. pathways of antibody affinity maturation). This is relevant across the spectrum of disease, including cancer, infectious disease and auto-immunity. It is also relevant to more fundamental questions of characterising mechanisms of protein binding.

What we know

We have developed a wet lab pipeline for high-throughput sequencing of antibody and T cell receptor repertoires and a bio-informatic pipeline for data analysis. Both of these are continually expanding in capacity. This allows us to annotate sequences, characterise diversity and build statistical models to measure differences between immune repertoires.

An example application discovered an antibody sequence motif whose presence we show biases the developmental fate of B cells. We have structurally mapped the motif to generate a hypothesis about the mechanism of B-cell receptor signalling. In a second example we have mapped receptor diversity in healthy vs. disease states.

What we need

Clinical and pre-clinical models in which to assess immune response. Wet lab work to express antibody sequences for testing of hypotheses generated by sequencing. Structural studies to help solve the sequence-to-structure bottleneck.

We are also interested in computational and mathematically oriented collaborators interested in developing models or computationally fitting models to big data sets.

Discovery of potent inhibitors of the epigenetic cancer target PRMT4

Kamilla Meyer[1] & Brian Lohse[1].

[1]Department of drug design and pharmacology, University of Copenhagen.

Brief description of research area
The PRMT4 protein (CARM1) regulates a number of cellular processes, including transcription, mRNA splicing, cell cycle progression, and DNA damage response trough epigenetic mechanisms. PRMT4 levels are increased in castration-resistant prostate cancers, 75% of colorectal cancers, and in aggressive breast tumors. Absence of PRMT4 methyltransferase activity of p300-R2142, which is required for its interaction with glucocorticoid receptor-interacting protein 1 (GRIP1) to signal DNA damage, results in loss of cell cycle arrest. The overexpression of PRMT4 activates a multitude of oncogenic pathways and promotes a favorable microenvironment for tumor growth and metastasis. To this date, few PRMT4 inhibitors have been discovered, among which EPZ011652 is an example. Unfortunately, EPZ011652, similarly to other discovered PRMT inhibitors, unselectively inhibits both PRMT1, -4, -6, and -8. A new approach could thus be to target the surface for allosteric sites of the PRMTs to obtain selective inhibitors. This can be achieved using phage display selection, which is the leading biotechnology for isolating highly specific and strong binders out of a billion peptides, nano- or antibodies. It is based on a M13 phage vector, modified for pentavalent display of ligands on its surface toward the coated protein (epigenetic target). After several washing steps, unbound and weak binders will be outcompeted by strong binders, making the pool of strong binders increase. Then, hits can be isolated and tested on Enzyme-linked immunosorbent assay (ELISA) for reconfirmation of binding to the target.

What we know
Two libraries consisting of billions of 16-mer and 19-mer linear peptides were screened against PRMT4 using the phage display technology. Nineteen peptides were discovered as PRMT4 binders based on ELISA assays, and the six most potent binders were chosen for further competition studies between the protein and the peptide displaying phages. The DNA of two peptides was successfully sequenced and the amino acid sequenced determined.

What we need
The two linear peptides are to be synthesized and tested in competition assays, their affinity is to be determined, and *in vivo* assays in human kidney tumorigenic HEK293 cells are to be performed. A competition assay between the peptide and protein will be optimal to determine the IC_{50} value, and affinity assays such as ITC will determine the K_D value of the peptides. The most potent binders will be fluorescently labeled with “TMR” and investigated *in vivo* using direct immunofluorescent assay in HEK293. This will determine its selectivity of the peptide inside the cell, and whether it is by a direct or secondary interaction, it inhibits the target or cancer cell growth. This will give ideal information to start a peptidomimetics study.

Exploring the secretomes of starch degrading fungi

Laura Nekiunaite[1], Magnus Øverlie Arntzen[2], Gustav Vaaje-Kolstad[2], Birte Svensson[1] and Maher Abou Hachem[1]

1. Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.
2. Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (UMB), P. O. Box 5003, N-1432 Ås, Norway.

Starch is one of the most abundant renewable biopolymers in nature. Each year more than 2 billion tons of starch crops are harvested globally, making it an attractive resource for industrial applications [1,2]. Most applications of starch require the disruption of native granules or starch modification. Thus, developing efficient enzymes that can hydrolyse and modify native starch granules is of interest. The properties of starch granules, especially crystallinity, which varies between 15 to 45%, make complete enzymatic depolymerisation of starch challenging depending on botanic origin and processing [3].

In contrast to lignocellulose matrices [4], much less effort has been imparted on discerning the enzyme cocktails deployed by fungi, both with respect to class, composition and proportion, for the degradation of starch. This insight is likely to have impact on the efficiency of industrial starch processing.

Fungi are renowned for producing an arsenal of carbohydrate active enzymes (CAZymes) [5] to harvest energy from various glycans. The main focus of the present study is to examine the degradation machinery of starch by filamentous *Aspergillus* spp., which exhibit tremendous ecological, biological and metabolic diversity and are used industrially for recombinant enzyme production.

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What we know

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

What we need

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

Discovery of human antibodies against snake venom phospholipase A_2s

Line Præst Lauridsen[1] , Andreas H. Laustsen[2], Mikael Rørdam Andersen[1], and Brian Lohse[2]

[1]*Department of Systems Biology, Technical University of Denmark*
[2]*Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen*

Brief description of research area
Snakebite is one of the world's most neglected tropical diseases, with an estimated 5.5 million bites per year, which results in 125.000 deaths. The only current treatment for snakebite envenoming is antisera derived from the blood of immunized mammals (typically horses). These antisera are expensive to produce and carry a high risk of causing hyper-allergic reactions in human recipients, due to their heterologous origin.

What we know
Venom of the Olive sea snake (*Aipysurus laevis*) is composed by short neurotoxins and phospholipase A_2s (PLA_2). Using phage display selection, we have discovered a number of human scFv binders, which are currently being sequenced, and their cross-reactivity patterns investigated by ELISA-based methods.

What we need
Currently, we are at a point, where we need to produce soluble scFv fragments in larger scale in order to perform affinity studies using isothermal titration calorimetry (ITC) or surface plasmon resonance (SPR). We furthermore intend to expand our investigation of scFv cross-reactivity to PLA_2s from other snake species. We hope to develop human scFvs that may broadly neutralize snake venom PLA_2s across snake genera.

Identification and characterization of a novel fungal ascomycete laccase

Majid Haddad Momeni[1], Paolo Bollella[2], Lo Gorton[2], Maher Abou Hachem[1]

[1] Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Elektrovej, 2800 Kongens Lyngby, Denmark
[2] Department of Analytical Chemistry/Biochemistry, P.O. Box 124, 221 00, Lund, Sweden

Brief description of research area

Infection of plants, especially major crops used in food and fodder, by pathogenic fungi has devastating economic effects globally.[1] This pathogenesis is typically associated with secretion of cell-wall deconstructing hydrolases, but also with an oxidative burst that is partially attributed to a large family of enzymes referred to as multi-copper oxidases[2] (MCOs).

Laccases (EC 1.10.3.2) are MCOs, which catalyze the oxidation of variety of phenolic substrates through a four-electron reduction of O_2 to H_2O. Filamentous Ascomycota and Basidiomycota, including plant pathogenic members, produce laccases assigned into auxiliary activity family 1 (AA1) in the CAZy database (http://www.cazy.org/). Beyond their possible roles in plant pathogenesis, fungal laccases are interesting candidates for utilization in biotechnological applications, such as food processing,[3] pulp-paper industries, bioremediation [4] and in bioelectrochemical systems.[5]

What we know

In this study, we explore the properties of a novel ascomycete laccase from a plant pathogenic fungus. This enzyme is modular consisting of a C-terminal catalytic module joint to the N-terminal starch binding module of family 20 (CBM20). In addition to this intriguing modularity, the enzyme displays a different amino acid sequence signature in a previously proposed specificity determinant located in the vicinity of the T1 copper-binding site.

What we need

The aim of this study is to reveal the binding and electro-catalytic properties of the enzyme. This first report on a modular laccases gives insight into the diversity of laccases with regard to substrate preference and the possible targeting of activity to distinct sites in infected plants. We are also interested in determining how the mutations of some amino acids in/around T1 site, might influence the enzymatic properties both in terms of kinetic and electrochemical parameters.

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Abstract no 13

Identification of citrullination sites specific for protein arginine deiminase (PAD) -2 and -4 in fibrinogen from synovial fluid of patients with rheumatoid arthritis

Mandvi Sharma[4], Anne-Christine Bay-Jensen[3], Birte Svensson[2], Per Hägglund[1].

1. Associate Professor; Protein and Immune Systems Biology; DTU Systems Biology.
2. Professor; Enzyme and Protein Chemistry; DTU Systems Biology.
3. Principle scientist; Head of Rheumatology; Nordic biosciences.
4. PhD; Protein and Immune Systems Biology; DTU Systems Biology.

Introduction
Rheumatoid arthritis (RA) is a multifactorial, complex, autoimmune disease. Protein citrullination, i.e. conversion of arginine residues into citrulline residues, is catalyzed by protein arginine deiminases (PADs), and is considered to be an important pathophysiological determinant in the ~80% of RA patients producing anti-citrullinated protein antibodies (ACPAs). Identification of citrullination sites on putative autoantigens is likely to enhance our understanding of PAD's substrate specificity.

Methods
Human fibrinogen from a plasma pool was citrullinated *in vitro* using human recombinant PAD2 and PAD4, and subjected to proteolysis using LysC. Soluble and non-soluble fractions in synovial fluid samples from RA patients were also subjected to LysC digestion after removal of IgG and serum albumin. The resulting peptides were subjected to LC-MS analysis on a Q-Exactive mass spectrometer using HCD fragmentation. Database search was performed using PEAKS 7.5 software (Bioinformatics Solutions, inc).

Preliminary Data
Using mass spectrometry we compared spectral characteristics and elution profiles for citrullinated peptides derived from fibrinogen contained in synovial fluid samples of RA patients, and fibrinogen citrullinated by the isoforms PAD2 or PAD4 *in vitro*. This enabled us to assign citrullination sites with high confidence. Fifty-two citrullinated sites were identified in total on the Aα (25 sites), Bβ (17 sites) and γ-chain (10 sites) of human fibrinogen. In synovial fluid of the patient samples Aα (10 sites), Bβ (8 sites) and γ-chain (3 sites) of human fibrinogen were identified. These included several novel sites on all three polypeptide chains that to our knowledge have not been previously reported.

Novel Aspect
Novel citrullination sites were identified in fibrinogen both of the *in vitro* treated samples and patient synovial fluid samples. Improvements in sample preparation and advancements in mass spectrometry is the need to develop a novel diagnostic and therapeutic tool against this chronic autoimmune disorder.

Exploring Xylan Degradation by the Human Gut Firmicutes *Roseburia intestinalis*

Maria Louise Leth and Maher Abou Hachem[1]

1. Dept. of Systems Biology, Technical University of Denmark, Lyngby, Denmark

Brief description of research area

The human gastrointestinal tract is colonized by a highly diverse and dynamic microbial community referred to as the human gut microbiota (HGM). Most diet derived complex glycans, which are non-digestible by the host, reach the lower part of the gastrointestinal tract intact and are metabolised by members of the HGM. Xylan is a major component of hemicellulose and one of the most abundant polysaccharides in the plant cell wall. This glycan is particularly relevant as a nutritional fibre in human diet as it comprises the main structural component in cereal crops e.g. in wheat, rye, barley and maize. The two dominant phyla in the gut are Firmicutes and Bacteroidetes and the primary xylan degraders in the gut are proposed to be *Roseburia intestinalis* and *Bacteriodes* species [1]. An increasing body of data is accumulating on the metabolism of complex glycans by members of *Bacteroides* [2]. However, insight into the xylanolytic system of *R. intestinalis* is currently lacking.

What we know

R. intestinalis has been reported to metabolize both soluble and insoluble xylans [3]. Its genome encode a modular extracellular endo-xylanases belonging to the glycoside hydrolase family 10 (GH10), according to the CAZy database of carbohydrate active enzymes. We have recombinantly expressed the GH10 endo-xylanase and its substrate preference and biochemical properties have been investigated. In addition to the GH10, the genome of *R. intestinalis* encodes several other glycoside hydrolases, esterases and transporters, which we believe is related to xylan degradation.

What we need

Characterization of the novel enzymes and transporters is necessary to elucidate the complete capture and break down of xylan by *R. intestinalis*. Insight from this study will promote our understanding of xylan metabolism by the underexplored Firmicutes species that is associated to a healthy microbiota. Growth studies with xylan degrading *Bacteriodes* species will in addition give insight in *R. intestinalis* hierarchy among xylan degraders in the human gut.

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Barley limit dextrinase inhibitor as backbone for design of proteinaceous inhibitors of industrial starch degrading enzymes

Marie Sofie Møller[1,2*], Sita Vaag Olesen[2], Birte Svensson[2], Ingemar André[1]

[1]*Department of Biochemistry and Structural Biology, Lund University, SE-221 00 Lund, Sweden.* [2]*Department of Systems Biology, Technical University of Denmark, DK-2800 Lyngby, Denmark. *msm@bio.dtu.dk*

Research area

Proteinaceous enzyme inhibitors have been shown to not only regulate activity, but also stabilize their target enzymes. The presented project focuses on design of inhibitors of a group of industrial enzymes, starch hydrolases. Inhibitors can be used for regulation of enzymes and recycling enzymes from industrial processes. A biochemically and structurally characterised limit dextrinase inhibitor (LDI; 13.4 kDa)[1] serves as template for computational protein design. LDI is from a family of α-amylase and serine-protease inhibitors, the cereal-type inhibitors. It binds to its target with picomolar affinity, and we have evidence for interaction hotspots in the complex with its target enzyme. LDI has a very high thermostability (T_m=97.4°C) making it an interesting starting point for design of binders for industrial processes.

The approach combines advanced computational methods including the software Rosetta and experimental characterisation of inhibitor variants using e.g. surface plasmon resonance and yeast surface display. Initial results have shown that LDI is a stable backbone for design, as mutation of up to 10% of all amino acid residues did not reduce the thermostability of the protein.

What we know

Protein (re)design using the Rosetta molecular modelling software suite; recombinant protein production in *Pichia pastoris* by high cell density fermentation; analysis of protein-protein interaction by surface plasmon resonance (SPR) and enzymatic methods.

What we need

Knowledge about design of the experimental setup for optimization of computationally designed protein binders by the combination of mutant libraries and yeast surface display combined with flow cytometry.

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

[1]Møller et al., *J. Biol. Chem.* **2015**, 290, 12614–12629.

Protein profiling of three distinct Chlamydia trachomatis growth forms

O Østergaard[1], A Olsen[2], PL Andersen[2], F Follman[2], NHH Heegaard[1], I Rosenkrands[2]

[1]Department of Autoimmunology and Biomarkers, Statens Serum Institut, Copenhagen, Denmark
[2]Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Denmark.

Brief description of research area
Proteome analysis of bacterial proteomes in order to obtain new knowledge about bacterial lifecycles

What we know
Infections with *Chlamydia trachomatis* is a frequent cause of sexually transmitted disease and may lead to infertility and other health issues if untreated. The bacterium is an obligate intercellular pathogen which needs host cells to replicate. Under normal growth conditions Chlamydia presents itself in two different morphological forms, the elementary body (EB) and the reticulate body (RB), during its developmental cycle. EBs represents an extracellular form with high infectious capacity, whereas the RBs are non-infective but show high metabolic and replicative activity. In addition, if exposed to stressful conditions the RB form can develop into an aberrant RB form termed ARB which represents a resting non-replicating but still metabolically active form.

What we need
Chlamydia often successfully evades the normal host immune response and is capable of establishing chronic infections. We need a better understanding of the Chlamydial lifecycle on a proteome level – in particular of the ARB form – to develop new vaccine candidates and strategies to fight and reduce infections with Chlamydia trachomatis. We strive to obtain this knowledge by LC-MSMS based quantitative proteome analysis of the three Chlamydial growth forms.

Scanning Electron Microscopy for Morphology and Membrane Damage Characterization of MRSA and *Pseudomonas aeruginosa* Challenged with Peptides Derived from Camel Milk Whey Protein

Ramona V. Mateiu[1](presenting author), Mahmoud Ibrahim[2], H. Jenssen[2] and J. Birkedal Wagner[1]
[1]DTU Cen, Center for Electron Nanoscopy, technical University of Denmark
[2]Department of Science, Systems and Models, Roskilde University

Brief description of research area

Within the last decade, bacterial resistance to conventional antibiotics has increased considerably. The increased resistance correlates with an increased consumption of antibiotics. Modifying already existing drugs is not always efficient as their mechanism of action often not change significantly compared to that of the parent drug, thus resulting in quick broad spectrum resistance development. Therefore, antimicrobial peptides and their synthetic analogues, which target directly the bacteria membrane, present a promising novel antimicrobial drug class.

We use scanning electron microscopy for visualization of cell morphology changes and membrane damage when the prokaryote is challenged with antimicrobial peptides extracted from camel milk whey protein. We quantify the information in the electron micrographs and use the findings as aid in understating the specific mechanism of action.

What we need

We would like to meet other people at DTU working with antimicrobial peptides.

Developing a microbial consortia for enzymatic conversion of keratin-rich slaughter-house waste into nutritional animal feed

Roall Espersen[1], Milena Gonzalo[2], Samuel Jacquiod[2], Waleed Abu-Alsud[2], Søren J. Sørensen[2], Jakob R. Winther[3], Per Hägglund[4], Birte Svensson[1]

[1] Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Elektrovej, Building 375, DK 2800 Kgs. Lyngby, Denmark
[2] Section of Microbiology, University of Copenhagen, Universitetsparken 15, Bld.1, 2100, Copenhagen
[3] Section for Biomolecular Sciences, University of Copenhagen, Ole Maaløes vej 5, Biocenter, DK 2200, Copenhagen, Denmark
[4]Protein and Immune Systems Biology, Department of Systems Biology, Technical University of Denmark, Building 301, DK 2800 Kgs. Lyngby, Denmark

Meat production from pigs is a resource heavy process as for example the pig feed often stems from soya beans grown in South America. Every part of the animal that is not used constitutes a protein food-chain lose, something that is not viable neither economically and environmentally. The goal of this project is to better harness resources from slaughterhouse waste e.g. keratin rich pig bristles and nails through microbial conversion. Using mass spectrometry and biochemistry based investigation techniques, the methods by which these organisms degrade the keratin fibers will be elucidated. Some of the protein families likely to be involved are special proteases (keratinases), also enzymes capable of reducing or otherwise breaking the disulfide bonds that are present in high abundance in hair and nails.

What we know
Protein chemistry and enzyme characterization; Amino acid analysis; Mass spectrometry and proteomics;

What we don't know
Growth of *Actinomadura* species

Soft Matter and Functional Interfaces

Roberto Ortiz[1], Esben Thormann[1]

1 Department of Chemistry, Kemitorvet, DTU, 2800 Kgs. Lyngby, Denmark.

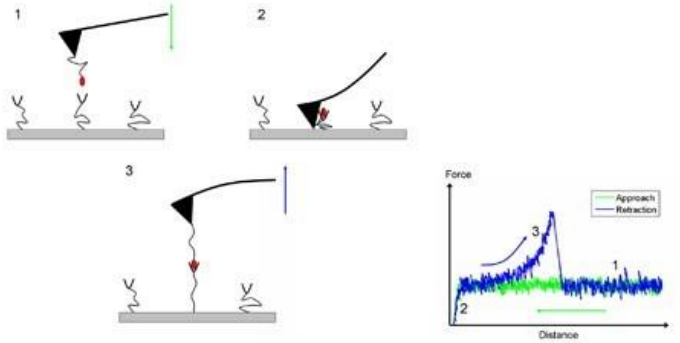
Brief description of research area
The research in the group is focused on fundamental and applied colloidal and surface chemistry and includes studies of intermolecular and surface forces, polymers, surface functionalization and characterization of surface structures. We are also designing thin films and polymer coatings with build-in functionalities such as anti-icing or anti-fouling properties and tuneable adhesion and lubrication.

Expertise
The group is highly skilled on characterization of soft matter on bulk and surfaces.
Surface characterization, AFM, colloidal probe AFM, polymers conformational studies, biosensors. Includes biomolecules immobilization, characterization and bioelectrochemistry (characterization of enzymes using electrochemistry).

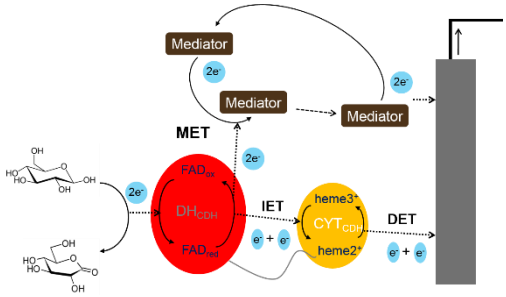
Techniques
Atomic force microscopy, quartz crystal microbalance with dissipation, calorimetry, electrochemistry, flow-injection techniques, dynamic light scattering, Z-potential, goniometer (contact angle).

What we need
We are interested in finding interesting proteins to study. AFM and QCM-D can be used to study conformational changes,

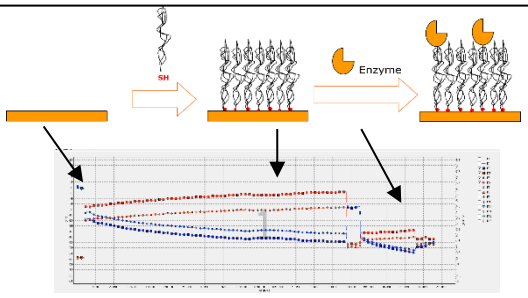
AFM for protein and polymer studies



Bioelectrochemistry, for the study of oxidoreductases



QCM-D for surface modification, characterization and conformational studies.



Characterization of native and modified milk protein (β-lactoglobulin) and their complexes with alginate

Saif Ullah[1], Seunghwan Lee[4], Emil G. P. Stender[2], Sanaullah Khan[1], Richard Ipsen[3], Birte Svensson[2] Kristoffer Almdal[1]

[1]Department of Micro- and Nanotechnology, Technical University of Denmark, Ørsteds Plads, building 345B, DK-2800 Kgs. Lyngby, Denmark.
[2]Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Elektrovej, building 375, DK-2800 Kgs. Lyngby, Denmark.
[3]Department of Food Science, Dairy, Meat and Plant Product Technology, University of Copenhagen, Rolighedsvej 30, 1958 Frb. C. Denmark.
[4]Department of Mechanical Engineering, Technical University of Denmark, Produktionstorvet, Building 425, 2800 Kgs. Lyngby, Denmark.

Brief description of research area
Proteins and polysaccharides are the two main macronutrients in the food and knowledge of how these structural entities interact is the key to understanding their functionality and unltimately to understanding digestion process. In this work we studied an animal's origin low molecular weight whey protein β-lactoglobulin (βLG), found in the cow's milk and a plant's origin polysaccharide sodium alginate extracted from seaweed. We characterized native and modified βLG and their interactions with alginate in solution.

What we know
Electrostatic interactions between oppositely charged macromolecules are one of the major driving forces that bind them together. We have modified native βLG, resulting in a comparatively high positive surface charge at pH 7 and the degree of protein modification was assessed by MALDI-TOF. To characterize the interactions between alginate and βLG, we performed dynamic light scattering (DLS), zeta potential and circular dichroism (CD). DLS showed that average hydrodynamic size of modified βLG was approximately same as that of native βLG and zeta potential values changed from high negative (native βLG at pH 7, -20 mV) to either less negative (6 residues modified βLG at pH 7, -9 mV) or completely positive (10 residues modified βLG at pH 7, 3 mV). βLG-alginate interactions were studied at pH 4 and 7. Circular Dichroism (CD) showed slight conformational changes of native and modified βLG after complexes formation.

What we need
To investigate how β-lactoglobulin interacts with alginate and what is the structural mechanism of these complex assemblies.

Development of peptidic anti-dendrotoxins

Saioa Oscoz1, Andreas H. Laustsen2, Mads H. Clausen3, and Brian Lohse2

1Department of Systems Biology, Technical University of Denmark
2Department of Drug Design and Pharmacology, University of Copenhagen
3Department of Chemistry, Technical University of Denmark

Brief description of research area
The black mamba (*Dendroaspis polylepis*) is one of the most feared and dangerous snakes in the world, and its bite has a very high mortality and morbidity rate. Dendrotoxins, the most abundant and some of the most toxic components present in black mamba venom, target potassium channels in neuronal tissue, leading to hyper-excitability in victims and prey. Blockage of the potassium channels can lead to respiratory paralysis and eventually death. Early administration of appropriate antivenom is the only effective snakebite therapy to date. However, current antivenoms are still being produced by the very laborious and expensive traditional animal immunization techniques, leading to severe side effects in human recipients due to their heterologous nature. In contrast, novel approaches based on synthetic or recombinant antivenoms may offer an alternative solution, saving cost, limiting side effects, and providing more effective neutralization of snake venom.

What we know
Peptide-based antitoxins against Dendrotoxin B were previously discovered through phage display selection. The peptidic hits were able to cross-react with toxins from different snake species in ELISA-based assays. Competition assays and ITC experiments were also performed, but so far strong binding has not been detected.

What we need
Currently, we are working on measuring the affinity of the peptidic antitoxins in order to rank how well these bind to the dendrotoxins. This will enable us to select the promising toxin binder, which we intend to test in two-electrode voltage clamp experiments to determine if toxin binding translates to toxin inhibition. Hopefully, these experiments will help guide future optimization of our peptidic antitoxins. However, we are in need of a more effective method for determining affinity.

Solution scattering studies of bacterial exopolysaccharides and their interactions with β-lactoglobulin

Sanaullah Khan[1], Johnny Birch[2], Saif Ullah[1], Marie-Rose Van Calsteren[3], Pernille Harris[4], Kristoffer Almdal[1], Birte Svensson[2]

[1]Department of Micro- and Nanotechnology, Technical University of Denmark, Ørsteds Plads, building 345B, DK-2800 Kgs. Lyngby, Denmark.
[2]Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Elektrovej, building 375, DK-2800 Kgs. Lyngby, Denmark.
[3]Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, Saint-Hyacinthe, Quebec J2S 8E3, Canada.
[4]Department of Chemistry, Technical University of Denmark, building 206, DK-2800 Kgs. Lyngby, Denmark.

Brief description of research area

Exopolysaccharides (EPSs) are secreted by a vast variety of microorganisms, including yeasts, moulds, microalgae, Gram-positive and Gram-negative bacteria. Bacterial exopolysaccharides have recently attracted considerable attentions due to their improved physical and rheological properties in fermented milk products. In this study, we characterized EPS and their interactions with β-lactoglobulin in solution.

What we know

Despite knowledge of high resolution NMR solution structures for repeating units (dp6) of EPSs, no structures for full-length of EPS have been reported up to now. Here, we determined the solution structures for three EPSs by X-ray solution scattering and constrained scattering modelling. To characterize the interactions between EPS and β-lactoglobulin, we performed dynamic light scattering (DLS) and X-ray solution scattering with β-lactoglobulin titrated with EPSs. DLS showed that β-lactoglobulin formed large particles with average diameter increased from 6 nm to 2.5 μm. SAXS radius of gyration (R_G) of β-lactoglobulin in the presence of EPSs increased significantly from 2.3 to ~16 nm, and the maximum lengths of β-lactoglobulin increased from 7.5 to 50 nm, confirming that large particles had formed.

What we need

To gain insight into how EPS binds to β-lactoglobulin, crystallography would be very useful to use.

Characterisation of non-histone N-ε acetylation in probiotics

Sita Vaag Olesen[1,2], Per Hägglund[2], and Birte Svensson[1]

[1]Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, 2800 Lyngby
[2]Center for Biological Sequence Analysis, Proteomics Core, Department of Systems Biology, Technical University of Denmark, 2800 Lyngby

Brief description of research area
N-ε lysine acetylation of non-histone proteins is a common posttranslational modification [1], and has been shown to be involved in cell metabolism [2]. It may therefore have the potential to function as biomarker for human diseases.

What we know
Acetylation and deacetylation of histones have, in humans, been shown to be involved in cancer [3,4] and studies indicate that deacetylation may also be related to the response to oxidative stress in a cell [5,6]. Lysine acetyltransferases (KATs) and lysine deacetylases (KDACs), as well as acetylation and deacetylation in general, have been investigated in various organisms such as *Escherichia coli*, *Bacillus subtilis*, *Mycobacterium tuberculosis*, and *Salmonella enterica* [7], including some 3D-structures. However, to our knowledge it has not been investigated in probiotics.

What we need
Production, purification, and characterisation of identified enzymes; Establishment of a mass spectrometry method for the investigation of the acetylome of *L. acidophilus* NCFM and for investigating the role of deacetylases in response to oxidative stress.

[1] Ouidir *et al.* (2015) *Proteomics,* [2] Yu *et al.* (2008) *J. Microbiol. Biotechn.,* [3] Lin *et al.* (1998) *Nature,* [4] Grignani *et al.* (1998) *Nature,* [5] Erjavec *et al.* (2007) *Proc. Natl. Acad. Sci. U. S. A.,* [6] Aguilaniu *et al.* (2003) *Science,* [7] Bernal *et al.* (2014) *New Biotech.*

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)

Transglycosylation reaction of bacterial *N*-acetyl hexosaminidases towards human milk oligosaccharide core structures

Triinu Visnapuu[1], Corinna Schiano di Cola[1], Takane Katayama[2], Aleksander Lie[3], Lars Haastrup Pedersen[3], Bettina Nonnemann[4], Branko Kokotovic[4], Peter Stougaard[5] and Birte Svensson[1]

[1]Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark; e-mail: trivi@bio.dtu.dk
[2]Graduate School of Biostudies, Kyoto University, Kyoto, Japan
[3]Section for Biotechnology, Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark
[4]National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark
[5]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark

β-*N*-acetyl hexosaminidases (βNAHAs; EC 3.2.1.52) are considered as enzymes with biotechnological interest due to their ability to produce bioactive and potentially prebiotic oligosaccharides. The capability of some βNAHAs to synthesize lacto-*N*-triose II [β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-Glc], a precursor of several human milk oligosaccharides, was recently shown (Appl Microbial Biotechnol 2015;99:7997-8009). Screening of bacterial species from various biotopes for the activities could give rise to novel βNAHA variants with transglycosylation activity.

In the present study, three new βNAHAs from various sources were isolated, recombinantly produced and characterized. Based on the substrate specificity, some of these enzymes preferably acted as *N*-acetyl glucosaminidases. Two of the enzymes showed transglycosylation activity and produced trisaccharide when using N'N"acetyl chitobiose as a donor and lactose as an acceptor. Despite the low identity between the enzymes at the protein sequence level, candidate conserved motifs probably involved in substrate binding and transglycosylation were identified.

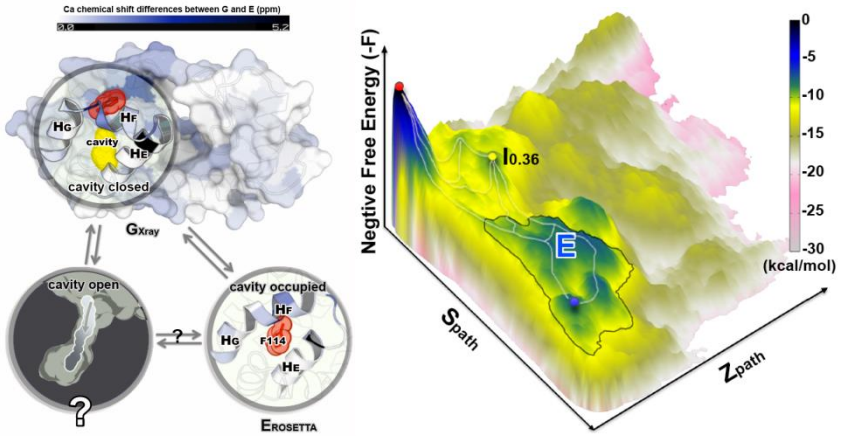
Additionally, the current work will benefit from expertise and techniques involving specific oligosaccharide detection, quantification and purification methods and genomic library construction to isolate other candidate βNAHAs.

Financial support
The Danish Council for Strategic Research grant OliGram. Design and gram scale enzymatic synthesis of human milk oligosaccharides.

Experiment Driven Simulations Provide a Global View on the Native-state Free Energy Landscape of a T4 Lysozyme Mutant

Yong Wang; Elena Papaleo and Kresten Lindorff-Larsen
Structural Biology and NMR Laboratory, Department of Biology, University of Copenhagen Email: wyongciac@gmail.com

While NMR and crystallography can readily provide structural information on the dominant conformations of proteins, it remains difficult to determine the structure of alternative, transiently populated states as well as understand the mechanisms by which they interconvert. The L99A variant of lysozyme from the T4 bacteriophage (T4L) has proven an excellent model system to understand protein structure and dynamics. The L99A mutant can bind hydrophobic molecules in an internal cavity, but the mechanism of access remains unknown and it has been speculated that a third hidden state must exist.



What we know

We have used several different methods by utilizing experimental information for enhancing molecular dynamics simulations to map the conformational landscape of the L99A mutant of T4L. Our simulations capture known conformations of T4L and provide insight into the mechanism of exchange between the states. Our results reveal hitherto unknown flexibility in the minor state of the protein that we show is compatible with NMR data. Accuracy is further supported by our ability to capture the effects of additional mutations that invert the populations of the major and minor states. Our simulations reveal the transient exposure of a tunnel towards the cavity, which we suggest represents a potential ligand binding pathway.

What we need

Experimental support of 1) the existence of the hidden third state with open tunnel that allows the entrance of the ligand; 2) the pathways that the ligand enter into and escape from the cavity.

Preparation of linear isomaltomegalosaccharide by *Gluconobacter oxydans* dextran dextrinase

Yuya Kumagai[1,2], Masayuki Okuyama[2], Atsuo Kimura[2], Maher Abou Hachem[1], and Birte Svensson[1]

[1]Department of Systems biology, Technical University of Denmark. [2]Research Faculty of Agriculture, Hokkaido University.

Brief description of research area

By definition, the megalosaccharide has DP (degree of polymerization) from 10 to 100. Generally, an individual function of carbohydrate is caused by its DP, monosaccharide unit, and linkage. Isomaltomegalosaccharide (L-IMS), formed by linear α-(1→6)-glucosyl structure, possesses high water solubility and beneficial functions of bioavailability of flavonoid glycoside. L-IMS is produced by the transglycosylation activity of Dextran dextrinase from *Gluconobacter oxydans* (DDase; EC 2.4.1.2), a secreted protein of *G. oxydans* which produced mainly dextran from maltooligosaccharides. The aim of our study is control of products length by molecular biology techniques.

What we know

We know the biochemistry and we can deal with carbohydrate relating enzyme and characterize megalosaccharides.

What we need

We need the knowledge and techniques of preparation of megalosaccharides efficiently.

List of Participants

First name	Surname	Title	Affiliation	E-mail	Poster No
Ana Rita	Colaço	PhD Student	DTU Chemistry	anaco@kemi.dtu.dk	1
Anders	Varming	Master Student	University of CPH	Hmp733@alumni.ku.dk	
Anette	Henriksen	Principal Scientist	Novo Nordisk A/S	athx@novonordisk.com	
Anna	Leuchsenring	PhD Student	DTU Vet	ableu@vet.dtu.dk	
Anna	Rosengren	Dr	Lund University	anna.rosengren@biochemistry.lu.se	2
Anne	Blicher	Head Laboratory Technician	DTU-Systembiologi	ab@bio.dtu.dk	
Ashish	Thakur	Visiting Intern	DTU Nanotech	ashtha@nanotech.dtu.dk	
Avishek	Majumder	Mr	Einar Willumsen	avishek.majumder@gmail.com	
Birte	Svensson	Professor	Systembiologi	bis@dtu.dk	
Bo	Jørgensen	Associate Professor	DTU National Food Institute	bojo@food.dtu.dk	
Camilla Aarup	Kristensen	Student	DTU	s133533@student.dtu.dk	
Charlotte B.	Madsen	Senior Researcher	DTU-FOOD	charm@food.dtu.dk	
Chris Juul	Hedegaard	Postdoc	DTU VET	cjhe@vet.dtu.dk	
Christine	Skovbjerg	PhD Student	Department of Systems Biology, DTU	caesk@bio.dtu.dk	
Corinna	Schiano di Cola	Ms.	DTU Systems biology	corinna.schiano@gmail.com	
David	Cannella	Postdoc	University of Copenhagen	dac@ign.ku.dk	

Elisabete	Xufre	Ms	DTU Systems Biology	lisa.xufre@gmail.com	
Emil G. P.	Stender	PhD Student	Technical University of Denmark	emigst@bio.dtu.dk	3
Flemming	Jessen	Senior Researcher	DTU Food	fjes@food.dtu.dk	
Folmer	Fredslund	PhD	Department of Chemistry, University of Copenhagen	folmer@chem.ku.dk	4
Giovanni	Rizzi	Dr	DTU Nanotech	giori@nanotech.dtu.dk	
Günther	Peters	Associate Professor	DTU Chemistry	ghp@kemi.dtu.dk	
Hans Mølager	Christensen	Associate Professor	DTU Chemistry	hemc@kemi.dtu.dk	
Hasan Ufuk	Celebioglu	PhD Student	Technical University of Denmark	hucel@bio.dtu.dk	5
Heidi Asschenfeldt	Ernst	Postdoc	Department of Chemistry, University of Copenhagen	hae@chem.ku.dk	
Henrik	Bohr	Dr	DTU Kemi	hbohr@dtu.dk	
Henrik	Flindt		Copenhagen University		6
Henrik	Stalbrand	Professor	Biochemistry and Structural Biology, Lund university	henrik.stalbrand@biochemistry.lu.se	
Jakob	Bohr	Professor	DTU Nanotech	jabo@nanotech.dtu.dk	
Jens O.	Duus	Professor	DTU Chemistry	jduus@kemi.dtu.dk	
Jeppe	Tams	Science Manager	Novozymes	jwta@novozymes.com	
Jesper	Vind	Senior Science Manager	novozymes	jvi@novozymes.com	
Jesper	Ferkinghoff-Borg	Associate Professor	BRIC	jesper.borg@bric.ku.dk	
Johnny	Birch	PhD sStudent	Enzyme and protein chemistry group (DTU)	birch@bio.dtu.dk	7

Joseph	Kaplinsky	Researcher	Department of Micro- and Nanotechnology DTU Nanotech	jkap@nanotech.dtu.dk	8
Julia	Tanassi	Laboratory Technician	Statens Serum Institut	jut@ssi.dk	
Justyna	Bahl	Ms	Statens Serum Institut	jmc@ssi.dk	
Kaj Frank	Jensen	Professor Emeritus	University of Copenhagen	kajfrankjensen@gmail.com	
Kamilla	Meyer		Copenhagen University	lcs879@alumni.ku.dk	9
Kasper	Tidemand	PhD Student	DTU Kemi	kdati@kemi.dtu.dk	
Kresten Jon Kromphardt	Olsen	PhD Student	DTU Systemsbiology	krjko@dtu.dk	
Kristoffer	Almdal	Professor	DTU Nanotech	kral@nanotech.dtu.dk	
Laura	Nekiunaite	Student	DTU Systems Biology	lanek@bio.dtu.dk	10
Leila	Lo Leggio	Associate Professor	KU Chemistry	leila@chem.ku.dk	
Lene	Lange	Professor	DTU-KT	lenl@kt.dtu.dk	
Line Abildgaard	Ryberg	PhD	DTU Chemistry	linear@kemi.dtu.dk	
Line Præst	Lauridsen		Department of Systems Biology, Technical University of Denmark	lineplau@gmail.com	11
Maher	Abou Hachem	Associate Professor	DTU, Systems Biology	maha@bio.dtu.dk	
Majid	Haddad Momeni	Postdoc	DTU System Biology	mahad@bio.dtu.dk	12
Mandvi	Sharma	PhD Student	DTU Systems Biology	mansh@bio.dtu.dk	13
Maria	Leth	PhD Student	EPC, System Biology	malole@bio.dtu.dk	14

Marie Sofie	Møller	Postdoc	Technical University of Denmark/Lund University	msm@bio.dtu.dk	15
Marta	Delsoglio	Master Student	DTU	marta.delsoglio@edu.unito.it	
Martin	Dufva	Associate Professor	DTU Nanotech	martin.dufva@nanotech.dtu.dk	
Mathias	Gruber	PhD Student	Environmental engineering	Mgrub@env.dtu.dk	
Michael Skovbo	Windahl	Postdoc	Roskilde Universitet	windahl@ruc.dk	
Morten	Ejby	Postdoc	DTU	me@bio.dtu.dk	
Nicolai	Johansen	PhD Student	Niels Bohr Institute	nicolai.johansen@nbi.ku.dk	
Niels	H.H. Heegaard	Director	Statens Serum Institut	nhe@ssi.dk	
Ole	Østergaard		Afdelingen for Autoimmonologi og Biomarkører	ooe@ssi.dk	16
Per	Hägglund	Associate professor	DTU Systems biology	ph@bio.dtu.dk	
Pernille	Jensen	Postdoc	Københavns Universitet	pernille.jensen@sund.ku.dk	
Pernille	Harris	Associate Professor	Technical University of Denmark	ph@kemi.dtu.dk	
Pernille	Sønderby	PhD Student	DTU Chemistry	perso@kemi.dtu.dk	
Pernille Rose	Jensen	Associate Professor	DTU elektro	peroje@elektro.dtu.dk	
Peter	Fristrup	Associate Professor	DTU Chemistry	pf@kemi.dtu.dk	
Peter	Busk	Scientist	Tailorzyme and DTU	pbus@kt.dtu.dk	
Peter	Heegaard	Professor	DTU Vet	pmhh@vet.dtu.dk	
Ramona V	Mateiu	PhD	DTU Cen	ram@cen.dtu.dk	17
Rasmus	Frandsen	Assistant Professor	DTU - SysBio	rasf@bio.dtu.dk	

Roall	Espersen	PhD Student	DTU	roes@bio.dtu.dk	18
Roberto	Ortiz	Postdoc	DTU Chemistry	robor@kemi.dtu.dk	19
Saif	Ullah	Postdoc	DTU Nanotech	saul@nanotech.dtu.dk	20
Saioa	Oscoz	Student	Systems Biology, DTU	saio_jere@hotmail.com	21
Sanullah	Khan	Dr.	DTU Nanotech	sank@nanotech.dtu.dk	22
Sebastian	Meier	Senior Researcher	DTU Chemistry	semei@kemi.dtu.dk	
Signe	Pedersen	Student	DTU	s133516@student.dtu.dk	
Sindrila	Dutta Banik	Postdoc	DTU	siduba@kemi.dtu.dk	
Sine	Larsen	Professor	Department of Chemistry, University of Copenhagen	sine@chem.ku.dk	
Sine	Reker	Associate Professor	VET	sirha@dtu.vet.dk	
Sita	Vaag Olesen	PhD Student	DTU Systems Biology, Enzyme and Protein Chemistry	svaol@dtu.dk	23
Susan	Andersen	PhD Student	DTU Systems Biology	sjes@bio.dtu.dk	24
Thomas	Holberg Blicher	Science manager	Novozymes A/S	thb@novozymes.com	
Tiffany	Sztuk	Student		tiffanysztuk@gmail.com	
Tine	Frederiksen	PhD Student	DTU Chemistry	timf@kemi.dtu.dk	
Triinu	Visnapuu	Dr.	Technical University of Denmark	trivi@bio.dtu.dk	25
Ulla	Højen	Bachelor of Science Chemistry	DTU, Technical University of Denmark	s083556@student.dtu.dk	
Verena	Volf	Master Student	DTU	vevol@bio.dtu.dk	
Vibeke	Orlien	Associate Professor	Food, KU	vor@food.ku.dk	

Willy	Bjørklund		Thermo Fisher Scientific	Willy.bjorklund@thermofisher.com	
Yong	Wang		Structural Biology and NMR Laboratory, Department of Biology, University of Copenhagen	wyongciac@gmail.com	26
Yuya	Kumagai	Postdoc	Systems Biology	yuyaku@bio.dtu.dk	27

Speakers

First name	Surname	Title	Affiliation	E-mail
Hans Mølager	Christensen	Associate Professor	DTU Chemistry	hemc@kemi.dtu.dk
Leila	Lo Leggio	Associate Professor	KU Chemistry	leila@chem.ku.dk
Line Abildgaard	Ryberg	PhD	DTU Chemistry	linear@kemi.dtu.dk
Martin	Dufva	Associate Professor	DTU Nanotech	martin.dufva@nanotech.dtu.dk
Sine	Reker	Associate Professor	DTU VEt	sirha@dtu.vet.dk
Vibeke	Orlien	Associate Professor	Food, KU	vor@food.ku.dk

Protein.DTU Task Force

First name	Surname	Title	Affiliation	E-mail
Birte	Svensson	Professor	DTU Systems Biology	bis@bio.dtu.dk
Charlotte	Bernhard Madsen	Senior Scientist	DTU Food	charm@food.dtu.dk
Flemming	Jessen	Senior Researcher	DTU Food	fjes@food.dtu.dk
Günther	Peters	Associate Professor	DTU Chemistry	ghp@kemi.dtu.dk
Jenny	Emnéus	Professor	DTU Nanotech	jenny.emneus@nanotech.dtu.dk
Kristoffer	Almdal	Professor	DTU Nanotech	kral@nanotech.dtu.dk
Maher	Abou Hachem	Associate Professor	DTU Systems Biology	maha@bio.dtu.dk
Ole	Lund	Professor	DTU Systems Biology	lund@cbs.dtu.dk
Pernille	Harris	Associate Professor	DTU Chemistry	ph@kemi.dtu.dk
Peter	M. H. Heegaard	Professor	DTU Vet	PMHH@vet.dtu.dk