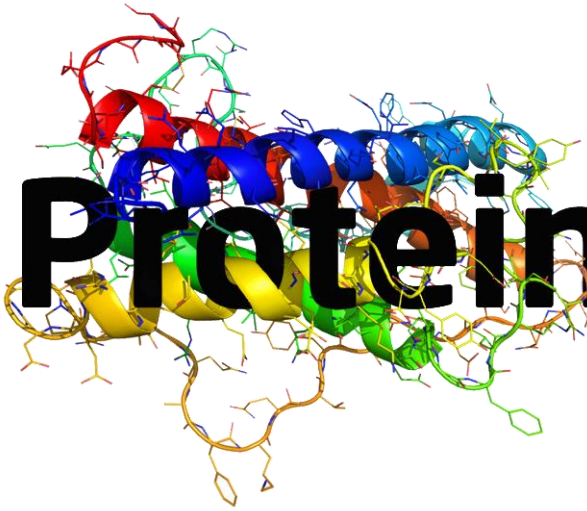
Future Trends in Protein Science

13th Workshop in



Protein.DTU

December 1, 2015

Future Trends in Protein Science
13th Workshop in Protein.DTU

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December 1, 2015
Building 101, Glassalen

08:30 – 09:00	**Arrival & Breakfast**
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09:10 – 09:40	***Proteins in a crowded world*** **Annalisa Pastore,** Professor, Maurice Wohl Insitute, King's College, England Chair: Maher Abou Hachem, Associate Professor, DTU Systems Biology
09:40 – 10:10	***Imaging molecular machines at the single-molecule scale*** **Sebastian Deindl**, Assistant Professor, Wallenberg Academy Fellow, Program for Computational and Systems Biology, Uppsala University, Sweden Chair: Kristoffer Almdal, Professor, DTU Nanotech
10:10 – 10:40	***Biological photovoltaics to harness solar energy*** **Hasan Kamrul**, PhD Student, Biochemistry and Structural Biology Department, Lund University, Sweden Chair: Jenny Emnéus, Professor, DTU Nanotech
10:40 – 11:05	**Coffee Break**
11:05 – 11:35	***Mining low abundance proteins in vegetable and animal foodstuff via combinatorial ligand libraries*** **Pier Giorgio Righetti**, Professor, Politecnico di Milano, Materials and Chemical Engineering "Giulio Natta", Milano, Italy Chair: Flemming Jessen, Senior Scientist, DTU Food
11:35 – 12:15	***The importance of polyvalent protein binding in immunology*** **Thomas Vorup-Jensen**, Professor, Department of Biomedicine, Aarhus University Chair: Peter M.H. Heegaard, Professor, DTU Vet
12:15 – 12:45	**Lunch and poster viewing**

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12:45 – 13:30	**Poster Session, coffee/tea, soft drinks & cake** Chair: Kristoffer Almdal, Peter Heegaard, Jenny Emnéus
13:30 – 14:00	***Exploring bacterial pathogenicity with proteomics*** **Julia Chamot-Rooke,** Directeur de Recherche, Head of Structural Mass Spectrometry and Proteomics Unit, Institut Pasteur, Paris, France Chair: Per Hägglund (Associate Professor, DTU Systems Biology)
14:00 – 14:30	***How does nature make glycosidic bonds*** **Carme Rovira**, ICREA Research Professor, Experimental Sciences & Mathematics, Universitat de Barcelona, Spain Chair: Günther Peters, Associate Professor, DTU Chemistry
14:30 – 15:00	***An enzyme producing rocket fuel: The inner workings of the hydrazine synthase multiprotein complex*** **Andreas Dietl**, PhD Student, Department of Biomolecular Mechanisms, Max Planck Institute for Medical Research, Heidelberg, Germany Chair: Pernille Harris, Associate Professor, DTU Chemistry
15:00 – 15:20	**Coffee Break**
15:20 – 15:50	***Why reinvent the wheel? Designing new antibodies and enzymes using natural backbone fragments*** **Sarel Fleishman**, Principal Investigator, Computational protein design and engineering, screening, Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel Chair: Ole Lund, Professor, DTU Systems Biology
15:50 – 16:20	***Co-translational protein folding inside and outside the ribosome exit tunnel*** **Gunnar von Heijne**, Professor, Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Sweden Chair: Birte Svensson, Professor, DTU Systems Biology
16:20 – 17:30	**End of day -"time for a beer" & posters**

Speaker Abstract

09:10 - 09:40

Proteins in a crowded world

Annalisa Pastore, Maurice Wohl Institute, King's College London

After more than 60 years of biochemical and biophysical studies, we have become accustomed to think that proteins are highly purified entities acting in isolation and freely diffusing until they find their cognate partners. This is certainly true in our in vitro experiments designed to reproduce these conditions. From them we have gained a great deal of information.

More recently though, we have started realizing the complexity of the cell environment and have tried to consider alternative ways to investigate the intrinsic properties of molecules. In their natural milieu, proteins are surrounded by a mash of molecules of quite different chemical nature. Proteins thus live in 'crowded' environments which can, in principle, considerably modify their behaviour. On average, about 40% of the cellular volume is occupied by molecules. An additional layer of complexity comes also from the fact that biological macromolecules usually live and operate in extremely structured and 'confined' environments within the cell (endoplasmic reticulum, Golgi apparatus, cytoskeletal structures, etc). Several approaches have been developed in the attempt to take into account these factors both at the theoretical and experimental level.

The field, with all its challenges and limitations, will be reviewed in this lecture together with our view for future directions.

Speaker Abstract

09:40 - 10:10

Imaging molecular machines at the single-molecule scale

Sebastian Deindl, Assistant Professor, Wallenberg Academy Fellow, Program for Computational and Systems Biology

Single-molecule techniques are well suited to monitor the complex dynamics of molecular machines in real time, directly observe intermediate states, and dissect reaction pathways. We use of single-molecule fluorescence resonance energy transfer techniques, in conjunction with complementary biochemical assays and structural approaches, to study the mechanisms and regulation of various protein machines. Here, I will focus on one example and describe our work on ATP-dependent chromatin-remodelling enzymes that regulate access to genomic DNA.

Biological photovoltaics to harness solar energy

Kamrul Hasan, Department of Analytical Chemistry/Biochemistry and Structural Biology, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden kamrul.hasan@biochemistry.lu.se

The development of carbon neutral, sustainable and cost-effective energy is one of the greatest challenges of the 21st century. Solar energy is the most abundant among all renewable energy resources. The amount of solar energy radiating to the earth surface in one hour is greater than the entire annual global energy consumption[1]. Biological photovoltaics (BPV)[2] is emerging as a potential energy generating technology, where photosynthetic materials use sunlight to produce electrical power[3].

In this presentation the current state of art of this technology with recent development and future perspective will be presented. We have studied the photo-electrochemical communication of various photosynthetic materials for BPV, i.e., *Rhodobacter capsulatus* (purple bacteria)[4], thylakoid membranes (photosynthetic organelle)[5] *Lyptolyngbia* sp. (prokaryotic cyanobacteria)[6] as well as *Paulschulzia pseudovolvox* (eukaryotic algae)[7]. These findings could have potential implications in sustainable photosynthetic energy conversion and in light sensitive devices.

The Swedish Research Council and the European Commission financially supported this work.

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Mining low abundance proteins in vegetable and animal foodstuff via combinatorial ligand libraries

Pier Giorgio Righetti, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Polytechnic of Milan, Via Mancinelli 7, 20131 Milan, Italy - piergiorgio.righetti@polimi.it

Food allergy (FA) is an increasingly emergent issue in food science and technology. The number of patients that are interested has been steadily growing in the last ten years, making the FA issue of primary importance for clinicians as well as for food industry. This can be fully understood by considering that the most common injuring sources include, among others, milk, egg, wheat, soy, peanuts, tree nuts, fish and shellfish, all basic food matrices of the diet of the majority of human population worldwide, and are also the raw ingredients used by the industry for the production of practically all food preparations.

There might be alternatives, though, to allergenic foodstuff. A case in point is cow's milk, to which many new-borns are allergic and need replacements for survival. We have recently investigated the proteomes of alternate sources, notably donkey's and goat's milk, and detected a large number of low-abundance, previously not reported, species and discovered why both types of milk are non- or hypo-allergenic. More recently, we have analysed the hidden proteome of bovine colostrum and tabulated no less than 1786 unique gene products, an extremely large number as compared to what reported in the latest literature (barely 400 species). Protein networks were then created on the basis of biological functions or health claims as input. A set of 93 proteins involved in the wound healing process was identified which were clustered on the basis of their biological activities.

Time permitting, I will also report on the hidden proteomics of a trio of tropical fruits, namely banana, avocado and mango, in which as many as 1000 to 3000 unique gene products have been discovered and tabulated for the first time. Although some, not previously reported, allergens have been detected, we have also discovered proteins and peptides with positive health benefits. Some, there appears to be light at the end of the tunnel, after all!

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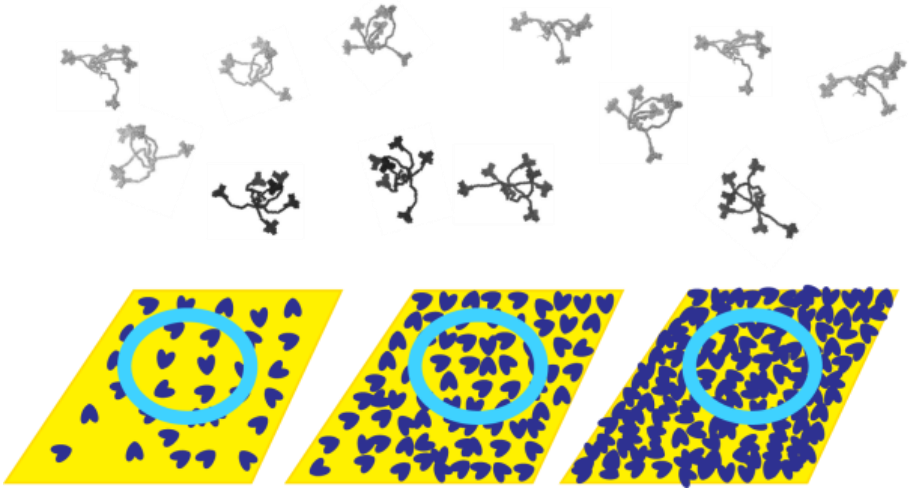
Speaker Abstract

11:35 - 12:15

The importance of polyvalent protein binding in immunology

Thomas Vorup-Jensen, MSc PhD DMSc[a,b,c]
[a]*Dept. of Biomedicine,* [b]*Interdisciplinary Nanoscience Center (iNANO),* [c]*MEMBRANES Research Center, Aarhus University, Aarhus, Denmark*
vorup-jensen@biomed.au.dk

In molecules of the immune system, a common structural element is the presence of multiple ligand binding domains. This enables strong, polyvalent interactions with targets such as bacterial cell surfaces and viral particles. From recent advances in understanding the thermodynamics of such interactions, I propose that innate immunity in part relies on the structural organization of ligand-presenting surfaces permitting the recognition of "topological patterns" to distinguish foe from friend.



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Exploring bacterial pathogenicity with proteomics

Julia Chamot-Rooke
Institut Pasteur, CNRS, Paris, France.

Recent advances in mass spectrometry (MS) technologies, protein and peptide separations as well as bioinformatics have made MS-based proteomics an indispensable research tool with the potential to broadly impact biology and medicine. There is an intense interest in applying proteomics to gain a better understanding of disease processes, identify new biomarkers for early diagnosis and discover novel protein targets for therapeutic interventions or drug development. As a result, various proteomics approaches that take advantage of the vast amount of genomic data now available on many pathogenic species, have been developed to study infectious diseases.

Top-down proteomics is an emerging technology based on the analysis of intact proteins using high-resolution mass spectrometry. This is in contrast to the better-established bottom-up strategy, which relies on the analysis of peptide fragments obtained after enzymatic digestion. A great advantage of top-down proteomics is its ability to address protein variations and characterize proteoforms arising from alternative splicing, allelic variation, or post-translational modification (PTM). [1] Top-down proteomics provides information closely connected to complex disease phenotypes and thus is of great interest for clinical applications.

This lecture will show how top-down proteomics can be used for exploring bacterial pathogenicity and in particular characterize bacterial proteins involved in the virulence of *N. meningitidis*. [2-3]

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Speaker Abstract

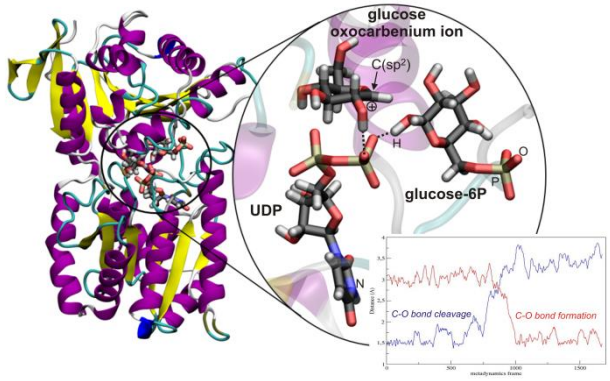
14:00 - 14:30

How does nature make glycosidic bonds

Carme Rovira ICREA Research Professor, Experimental Sciences & Mathematics, Universitat de Barcelona, Spain

a, Departament de Química Orgànica, *Universitat* de Barcelona, Martí i Franquès 1, 08028 Barcelona,
b, Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08018 Barcelona.
c.rovira@ub.edu

One of the great scientific challenges of our time is to find new molecules as potential new drugs to fight disease. Carbohydrate-active enzymes are the focus of enormous interest due to the critically important roles that complex glycans play in health and disease. Nature has created a several ingenious methods to form glycosidic bonds in carbohydrates, via the action efficient enzymes named glycosyltransferases. Here we will show our work of the last few years on the prediction of catalytic molecular mechanisms of these enzymes, using quantum mechanics/molecular mechanics (QM/MM) and metadynamics methods.



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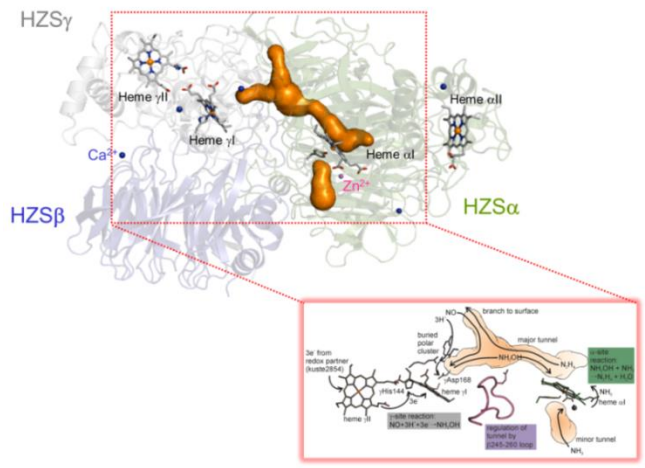
An enzyme producing rocket fuel: The inner workings of the hydrazine synthase multiprotein complex

Andreas Dietl[1], Christina Ferousi[2], Wouter J. Maalcke[2], Andreas Menzel[3], Simon de Vries[4,†], Jan T. Keltjens[2], Mike S.M. Jetten[2,4] , Boran Kartal[2] & Thomas R.M. Barends[1]

1) Department of Biomolecular Mechanisms, Max Planck Institute for Medical Research, 69120 Heidelberg, Germany
2) Department of Microbiology, Institute for Water and Wetland Research, Radboud University Nijmegen, 6525AJ Nijmegen, The Netherlands
3) Swiss Light Source, Paul Scherrer Institute, 5232 Villigen, Switzerland
4) Department of Biotechnology, Delft University of Technology, 2628 BC Delft, The Netherlands
5) Department of Biochemistry and Microbiology, Laboratory of Microbiology, Gent University, Gent, Belgium. †Deceased



Anaerobic ammonium oxidation (anammox) is a bacterial process that is an essential part of the earth's nitrogen cycle. Up to 50% of the nitrogen release from the oceans has been attributed to anammox and the process is now also frequently applied in waste water treatment plants. The anammox reaction combines nitrite and ammonium to form dinitrogen gas and water. Strikingly, hydrazine has been found to be a central intermediate in the anammox metabolism. This highly reactive compound has a very low redox potential (E_0'= -750 mV) and is therefore also used as a propellant in spacecraft. The enzymatic mechanism, however, by which hydrazine is biologically synthesized has so far remained elusive.

We determined the 2.7 Å resolution crystal structure of a hydrazine synthase (HZS) multienzyme complex (kuste2859-61) isolated from the anammox organism *Kuenenia stuttgartiensis* and characterized it both biophysically and spectroscopically. The HZS complex forms an elongated dimer of heterotrimers, each of which harbors two distinct c-type heme-containing active sites and an interaction interface for a redox partner. In addition, these active sites are connected by a system of tunnels. From the crystal structure a two-step mechanism for hydrazine synthesis can be proposed: first, nitric oxide is reduced to hydroxylamine in a three-electron reduction at the active site of the γ-subunit. Then, the resulting hydroxylamine diffuses through the tunnel to the second active centre in the α-subunit where it is subsequent condensed with ammonia, finally yielding hydrazine.

Reference

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Speaker Abstract

15:20 - 15:50

Why reinvent the wheel? Designing new antibodies and enzymes using natural backbone fragments

Dr. Sarel Fleishman, Assistant Professor, Weizmann Institute of Science, Israel

Computational protein design is a new field that uses computer algorithms to design new proteins and molecular functions. Recent progress in the field has generated the first designed enzymes, inhibitors, and binders by grafting functional amino acids on rigid, pre-existing natural backbones. Nevertheless the ability to design new backbones for function has remained elusive. We have therefore developed a strategy to design new backbones using fragments of homologous proteins and to optimise the sequence for stability and activity. This combinatorial backbone design strategy accesses an unprecedented space of potential backbones enabling high shape and chemical complementarity with the target molecules. We implemented this strategy to antibody structure prediction and design, generating high-affinity and specific antibody binders, and recently extended the strategy to enzyme design.

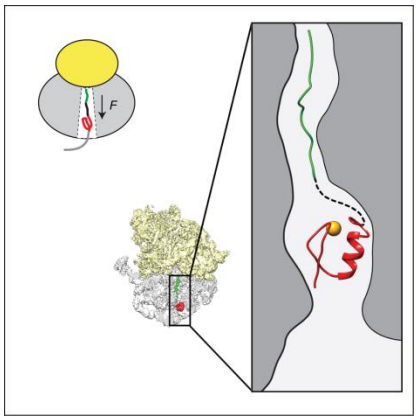
Speaker Abstract

15:50 - 16:20

Co-translational protein folding inside and outside the ribosome exit tunnel

Gunnar von Heijne
Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Sweden
gunnar@dbb.su.se

We have recently developed a technique based on the use of so-called translational arrest peptides to measure forces acting on a nascent chain during cotranslational processes such as membrane translocation and folding. In contrast to hard-core biophysical methods, the technique can be used both *in vitro* and *in vivo*. Recent results on cotranslational folding of cytoplasmic proteins will be presented.



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Redirecting photosynthetic reducing power towards bioactive natural product synthesis

Agnieszka Z. Nielsen, Maria Perestrello Ramos H de Jesus, Thiyagarajan Gnanasekaran, Silas Busck Mellor, Artur Jacek Wlodarczyk, Poul Erik Jensen

Copenhagen Plant Science Center, VILLUM Research Center for Plant Plasticity, Center for Synthetic Biology "bioSYNergy", Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark.

Photosynthesis provides the energy and carbon building blocks required for synthesis of a wealth of bioactive natural products which have potential uses as pharmaceuticals. Photosynthesis in plants or cyanobacteria provides ATP and NADPH as well as carbon sources for primary metabolism. Cytochrome P450 monooxygenases (P450s) in the plant endoplasmic reticulum (ER) are essential in the synthesis of many bioactive natural products, powered by single electron transfers from NADPH. P450s are present in low amounts and the reactions proceed relatively slowly due to limiting concentrations of NADPH. We have recently demonstrated that it is possible to break the evolutionary compartmentalization of energy generation, where an entire P450 dependent pathway can relocate to the chloroplast and use the reducing power generated by photosystem I in a light-dependent manner. This demonstrates the potential of transferring pathways for structurally complex high-value natural products and directly tapping into the reducing power generated by photosynthesis to drive the P450s using water as the primary electron donor. Current work establishes stable transgenic lines of both plants and cyanobacteria with multi-enzyme pathways expressed in the thylakoids and produce high-value bioactive compounds directly driven by light.

We explore different strategies to optimize product formation. One approach is scaffolding which aims at more efficient channelling of the substrate and intermediates. For this we utilize the protein binding properties of Tat proteins for building a modular synthetic scaffold that spatially recruits the necessary enzymes in a desirable manner. In another approach, we investigate the interaction of P450 enzymes with different ferredoxin proteins in order to optimise the electron transfer between photosystem I and the P450s.

We found differential interactions between chloroplast ferredoxins and ER-derived P450s, and these may improve partitioning of electrons from photosynthesis towards bioactive compound biosynthesis.

Expression and purification of TcdA and TcdB from *Clostridium Difficile*

Anna Bielecka Henriksen, Dasol Hwang & René Jørgensen.

Statens Serum Institut (mikrobiologi and infektionskontrol), KU-Science

Clostridium difficile infection is a common cause of health care–associated diarrhoea in industrialized countries, and the leading cause of intestinal infection related to antimicrobial drug consumption.

The virulence of this pathogen is attributed to two large exotoxins, toxin A and toxin B. The objective of this project is to purify the two toxins from *C. difficile*, structurally characterize the two toxins by using electron microscopy, to inactivate the two toxins using Hydrogen Peroxide, and evaluate the cytotoxicity on mammalian Vero cells with the purpose of developing a toxoid vaccine candidate.

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

A. R. 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[1].

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[2], Science[3], and Nature[4] have shown that acyl-lysine modifications other than acetyl are indeed biologically relevant[5,6], which have substantiated our main hypothesis considerably.
Several in vivo studies have described NADH as a weak competitive inhibitor for yeast sirtuins, however this had yet to be tested for mammalian sirtuins[7, 8]. As a starting point for this *in silico* study, we investigated the NADH inhibition of SIRT1, SIRT3 and SIRT5 using long Molecular Dynamics (MD) simulations[9] and Density Functional Theory (DFT) calculations[10]. Our results showed that the weak inhibition effect of NADH is highly correlated with the misalignment of the nicotinamide amide to the key residues interleucine and aspartate in the C pocket of the sirtuin.

What we need

The gap between known protein sequences and confirmed protein structures, functions and mechanisms, is immense. The increased complexity of the field has emphasized the need for more experimental data, to compare and validate the theoretical findings.

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Rapid selection and screening of a Cel5A random mutagenesis library for enhanced enzyme folding, solubility, and activity

Brian King[1], Jason Boock[2], Robert Conrado[2, 3], Matthew DeLisa[2, 4], and Donna Gibson[1,5]

1 Department of Plant Pathology and Plant-Microbe Interactions, Cornell University, Ithaca, NY, 14853, USA,
2 School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, 14853, USA
3 US Department of Energy, Advanced Research Projects Agency - Energy, Washington, D.C., 20585, USA
4 Department of Biomedical Engineering, Cornell University, Ithaca, NY, 14850, USA
5 US Department of Agriculture Agricultural Research Service, Robert W Holley Center for Agriculture and Health, Ithaca, NY, 14853, USA

Brief Description of Research Area
We have developed a method for screening protein libraries generated with random mutagenesis or other techniques. This method utilizes the folding "quality control" features of the bacterial twin-arginine translocation (Tat) pathway to eliminate poorly folded library members, and is combined with a functional enzyme activity assay. The end result are enzyme variants with improved cytosolic expression and retained enzymatic activity.

What We Know
Generation, selection, and screening of enzyme libraries; carbohydrate active enzymes; protein secretion

What We Need
Protein targets; extension/expansion/modification of the methodology; expertise on protein folding and thermodynamics

Characterization of V181A, a dimeric Hsp21 mutant by SAXS and CXMS

Cecilia Emanuelsson, Gudrun Rutsdottir, Katja Bernfur, Christopher Söderberg
Department of Biochemistry and Structural biology, Center for Molecular Protein Science, Lund University, PO Box 124 SE-221 00 Lund, Sweden.

Brief description of research area
Small heat-shock protein (sHsp) chaperones are important in the protein quality control system of the cell since they can interact with transiently or partially unfolded client proteins to avoid aggregation. The sHsp chaperones form oligomers that are exceptionally dynamic, which is implicated in their function. The mechanism of substrate recognition is not fully understood, and it remains unclear which parts of a partially unfolded client protein that interact with the sHsp.

What we know
To further understand the importance of the dynamic oligomers for these interactions we have created a dimeric V181A substitution mutant of our sHsp. The similarities and differences between the wildtype oligomer and the V181A mutant dimer were analyzed with two client proteins, malate dehydrogenase and citrate synthase, combining data collected from two structural approaches, Small Angle X-ray Scattering (SAXS) and crosslinking mass spectrometry (CXMS) using unlabeled and ^{15}N-labelled protein making it possible to monitor changes over time. Preliminary data suggest that the monomers and dimers of wildtype and V181A mutant are structurally similar since they can exchange subunits as detected by ^{14}N-^{15}N hybrid crosslinks. Yet the SAXS data suggested no formation of client protein-sHsp complexes which increase in size over time for the mutant V181A, which could only maintain client proteins in solution for a limited amount of time.

What we need
Complementarty methods to study conformations of oligomeric proteins and their complexes.

A novel class II Fic protein from *Clostridium difficile* reveals inhibitory motif-independent auto-adenylylation

Emil Dedic[1], Husam Alsarraf[1], Ditte Hededam Welner[1], Oleg I. Klychnikov[2], Paul J. Hensbergen[2], Ole Østergaard[3], Hans C. van Leeuwen[4] and René Jørgensen[1]
[1]Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark, [2]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands, [3]Department of Clinical Biochemistry and Immunology, Statens Serum Institut, Copenhagen, Denmark, [4]Department of Medical Microbiology, Center of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

Fic-domains in certain bacterial effectors have been shown to disrupt host cells by catalyzing the transfer of the AMP moiety from ATP onto protein targets.

What we know
We have characterized a newly identified Fic-domain protein, CdFic, from the pathogenic *Clostridium difficile*. CdFic is the first Fic-domain protein to be characterized from the group of Gram-positive bacteria. Crystal structures of CdFic show that the protein forms a dimer and has a classical all helical Fic fold belonging to the class II of Fic proteins. We also demonstrate that ATP has a different binding mode and reveal that CdFic auto-adenylylation activity is independent of the inhibitory motif contrary to any other known class I-III Fic-domain proteins. Finally, we show that ATP binding to CdFic triggers a disorder-to-order transition of a loop called, the flap.

What we need
Despite extensive attempts to elucidate the function of CdFic, we still have not identified the protein target and whether it acts as a virulence factor in *C. difficile*. The current status of the project will be presented at the meeting.

Characterizing interactions between alginates of different sizes and β-lactoglobulin

Emil G. P. Stender[1], Sanaullah Khan[2], Richard Ipsen[3], Maher Abou Hachem[1], Kristoffer Almdal[2], Peter Westh[4], 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. [4]Roskilde University – RUC – Department of Science, Systems and Models

Brief description of research area
Alginate is a linear anionic polysaccharide from brown algae that is of importance to the pharmaceutical and food industries because of its gelling and stabilizing properties. It consists of two C5 epimers mannuronate and guluronate arranged in homo or mixed blocks. Here the interaction of two alginates of different average polydispersity with β-lactoglobulin is characterized.

What we know
Alginate and β-lactolgobulin forms large insoluble particles when pH is below the pI of β-lactoglobulin. The interaction is likely ionic as β-lactoglobulin and alginate carry opposite charges when particles are formed as assessed by the ζ-potential. The particles formed have a hydrodynamic diameter of thousands of nanometer at pH 4 that decreases when pH is lowered as assessed by Dynamic Light Scattering. When assessed by ITC high molecular weight alginate interacts 11 times stronger than low molecular weight alginate at pH 3 and high molecular weight alginate binds 7 times the amount of protein corresponding to the difference in average polydispersity. At pH 4 the amount of protein bound per average alginate molecule increases but the strength of interaction decreases.

What we need
Particle formation at pH 4 and pH 3 is likely driven by different mechanisms. Quartz crystal microbalance analysis might be able to shed some light on this problem.
Mannuronate and guluronate blocks likely interact differently with β-lactoglobulin. Shorter poly-saccharides or oligosaccharides containing only one species could be used to asses this hypothesis.

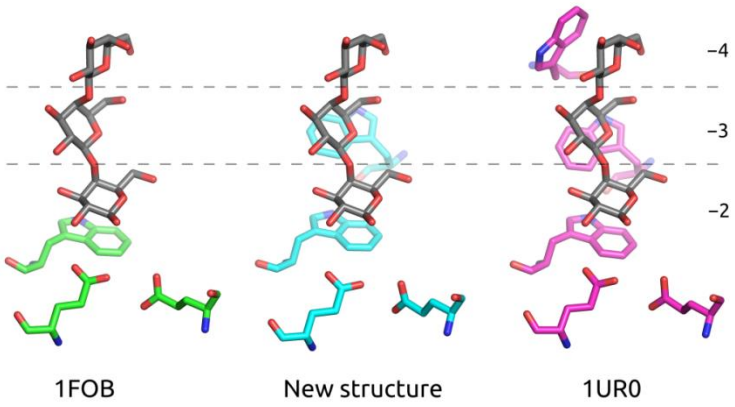
Structure of an archael GH53 β-1,4-GALACTANASE

F. Fredslund[a], J.-C. N. Poulsen[a], K. K. Rasmussen[a], K. Jensen[b], K. B. R. M. Krogh[b], L. Lo Leggio[a]

[a]Department of Chemistry, Universitetsparken 5, Copenhagen, 2100, Denmark
[b]Novozymes A/S, Krogshøjvej 36, Bagsværd, 2880, Denmark
leila@chem.ku.dk

Keywords: Glycoside hydrolase, thermostable, specificity, microfocus

β-1,4-galactanases are found in prokaryotic, eukaryotic and archaeal microorganisms, where they are thought to be involved in digestion of galactan side chains of plant pectins. They are found exclusively in the GH53 CAZY(1) family. Structures of GH53 enzymes from bacteria(2) (eg PDB code 1UR0) and fungi(3–5) (eg PDB code 1FOB) have previously been determined. Here we will present the structure of a β-1,4-galactanase from the archeon *Ignisphaera aggregans* DSM 17230 having a temperature optimum for activity at 90 °C.
A dataset was collected to 2.65Å resolution (I/sigma=1.43, CC(1/2)= 59.5 in outer resolution shell) from a



multiple needle at the microfocus beamline ID23-2 of the ESRF and solved with molecular replacment using MrBUMP. The final structure has an R_{work}/R_{free} of 20.8%/25.7% and 1 residue outside the allowed region of the Ramachandran plot. The implications of the structure for substrate/product specificity will be discussed.

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Studying back-exchange effects on protein analysis by hydrogen/deuterium exchange mass spectrometry

Gerard Comamala[1], Pernille F. Jensen[1], Kasper D. Rand[1]

1: Protein Analysis Lab, Section for Analytical Biosciences, Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

What we know

Hydrogen/Deuterium exchange mass spectrometry (HDX-MS) is a sensitive and high resolution technique capable of pinpointing changes in the structural dynamics of proteins. It is based on the hydrogen exchange phenomenon where protein hydrogens exchange out with solvent deuterium. The deuterium incorporation leads to a shift in mass that can measured by MS. Online desalting and pepsin digestion followed by UPLC separation of the resulting peptides make it possible to pinpoint the changes in mass with good spatial resolution.

In this case we focus on the effects of back-exchange on the technique.

What we need

- Basic knowledge of the NMR technique
- Other applications of mass spectrometry
- Everything related to protein glycosylation.

Functional quantitative proteomics: a rationale Omics of enzyme(s) activation/regulatory status deducted by proteomics quantitative detection of PTMs

Giuseppe Dionisio1, Adelina Rogowska-Wrzesinska2, Stasolla Claudio3
1) Department of Molecular Biology and Genetics, Faculty of Science and Technology, Aarhus University, Forsøgsvej 1, 4200 Slagelse (DK). Email: Giuseppe.dionisio@agrsci.dk;
2) Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M; e-mail: adelinar@bmb.sdu.dk
3) Full professor at Dept. Plant Science, University of Manitoba (Canada).E-mail: Claudio.Stasolla@umanitoba.ca

Brief description of research area
Quantitative proteomics performed by modern LC-MS/MS Orbitrap systems is an accurate quantitative high throughput screening of many proteins a time for a given organism. We have intension to focus on maize (*Zea mays*) proteome under anoxic conditions or Gibberella zea ear infections. Cysteine oxidation status is a common PTM that spans from irreversible to reversible status involving hence the enzymatic activity. Quantitative cysteine oxidation (**1**) could bring a functional aspect in quantitative proteomics giving for a selected time point a "freeze frame" of the enzymatic status as well. This aspect of quantitative PTMs will be evaluated for key proteins/enzymes during specific selected pathways that during different time point could represent a signal transduction if coupled with quantitative phosphorylation of Ser/Thr/Tyr and His.

What we know
Nitric oxide and peroxide signaling integration contribute to the oxidative status of signaling molecules during signal transduction. Fungal attach or flooding results in the alteration of these signal cascade mechanisms triggered by NO2 or H2O2 signalling (further mediated by salicylic acid or Jasmonic acid/ethylene). S-nitrosylation of NPR1, a general regulator of systemic acquired resistance (SAR) is also depending on the redox status kept also by thioredoxins. Fusarium is an hemibiotroph fungi and it has hence the capability to block or overcome the NO/H2O2 response and hence block the programmed cell death (PCD). The PCD is also known to occur in the "Aerenchyma" formation due to flooding in mays roots. We have developed a mays lines overexpressing or downregulating mays hemoglobin isogenes (**2**). Such plants have a modified response to NO signaling since plant hemoglobin (Pgb) is a natural scavenger of the NO due to the heme group. We would like to use these Pgb maize GMOs as control to verify the normal versus altered signal transduction pathway during anoxia and Fusarium infection.

What we need
We are looking for collaborative partners and funds for carrying on such "functional quantitative proteomics".

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Structural investigation of Tetanus and Diphtheria toxins and their formaldehyde-inactivated toxoids

Husam Alsarraf [1], Emil Dedic [2], Morten J. Bjerrum [3], Max P. Kristiansen [1], Jesper W. Petersen [1], René Jørgensen [1]

[1]Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark. [2]Institute of Molecular Biology and Biophysics, ETH, CH-8093 Zürich, Switzerland. [3]Department of Chemistry, University of Copenhagen, Copenhagen, Denmark.

Vaccination is one of the most cost-effective public health interventions to date, saving millions of lives and protecting countless of people from illness and disability. Statens Serum Institut produces tetanus and diphtheria vaccines based on toxoids, which are either chemically- or heat-inactivated toxins. The toxoids lack the potency, but preserve the toxins immunogenicity, a crucial criteria for vaccines function.

We investigate the structural integrity of Statens Serum Institute-produced tetanus and diphtheria vaccine toxoids. We apply an approach using a combination of spectroscopic and biophysical techniques, like size exclusion chromatography, circular dichroism, differential scanning fluorimetry and small angle x-ray scattering.

The results indicate that formaldehyde-inactivation preserves the secondary structure features and the overall shape of the toxoids. The toxoids are monomers in solution with a significant increase in size and they are far more stable compared to the respective toxins, even though the secondary structure content is unchanged. This is the first low resolution structural analysis presented on the full-length tetanus toxin confirming high degree of overall shape similarity to the structure of botulinum neurotoxin.

Design of protein and peptide self-assembly

Ingemar Andre
Department of Biochemistry and Structural Biology, Center for Molecular Protein Science, CMPS
Lund University

Brief description of research area
Large protein complexes carry out some of the most complex functions in biology. Such structures are often assembled spontaneously from individual components through the process of self-assembly. By coupling the powerful design template of self-assembly with computational protein design we can engineer new protein assemblies with custom-made structure and function.

What we know
Computational protein design and biophysical chemistry

What we need
Structural biology

Discovering novel glycan utilization loci in probiotic bacteria

Jens Vogensen[1], Quanhui Wang[2], Maher Abou Hachem[1], Siqi Liu[2], Birte Svensson[1]
jenvo@bio.dtu.dk

[1]*Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Denmark*
[2]Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, 100101, China

Consumption of host-indigestible glycans is essential for the maintenance of a healthy population of gut microbes. The thriving of probiotic bacteria, *Bifidobacterium* and *Lactobacillus* being the most commercially exploited strains, necessitates the intake of prebiotics[1]. βMannan-oligosaccharides (MOS) are one such potential prebiotic which is present in nature in different structures such as galactomannan and glucomannan[2]. Xyloglucan utilization loci have also recently been identified and characterized in several *Bacteroidetes* strains[3]. Not much is known about either MOS or xyloglucan utilization by probiotics, even less on their roles as prebiotics.

Identification of putative loci for MOS or xyloglucan utilization has been pursued through genome-mining in different strains of probiotic bacteria. Utilization of either glycan will be investigated in these probiotic taxa through bacterial growth assays. Identification of upregulated genes when the bacteria grow in media with or without oligosaccharides, including novel glycoside hydrolases (GHs) genes, will be carried out through transcriptomics and proteomics. These identified GHs will be functionally characterized including crystal structure determination. This investigation will broaden the knowledge of the different strategies gut microbes have evolved in the crowded and competitive gut environment to utilize varying carbohydrate sources. Preliminary results will be presented.

Acknowledgments
This research project is supported by the Sino-Danish Center (SDC) and Technical University of Denmark (DTU)

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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.

Brief description of research area

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 60–70 mg l^{-1} from *Lactobacillus rhamnosus* GG grown in skimmed milk medium. Molecular weights were determined by size exclusion chromatography (SEC) to 138±3–4683±442 kDa. By using surface plasmon resonance (SPR), binding of the HePS to *β*-lactoglobulin, *β-casein, κ-casein,* and heat treated *β*-lactoglobulin 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 *β*-lactoglobulin was supported dynamic light scattering. HePS oligosaccharide repeat units have been prepared to allow accurate studies of the protein-carbohydrate interactions by NMR spectroscopy and X-ray crystallography. 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 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 (α-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 can be expressed and purified and repeat unit oligosaccharides can be produced
- The HEPS show characteristic specificity in interaction with selected milk proteins.

What we need

- 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).

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Expression and characterization of novel fungal laccases

Majid Haddad Momeni[1], Maher Abou Hachem[1]
[1] Department of System biology, Technical University of Denmark

Brief description of research area
Laccases (EC 1.10.3.2) comprise a large family of multi-copper oxidases (MCO), which catalyze the oxidation of variety of phenolic substrates. Laccases are mostly produced by fungi, higher plants and to less extent insects and bacteria.

Fungal laccases, which are mainly encoded by filamentous fungi including Ascomycota and Basidiomycota, are classified as auxiliary activity family 1 (AA1) enzymes along oxidoreductases (EC 1.10.3.2) and ferroxidases (1.10.3.-) in the CaZY database (http://www.cazy.org/). Fungal laccases exhibit higher redox potentials (800 mV) as compared to plant or bacterial counterparts,[1] making them suitable candidates for utilization in a variety of biotechnological applications, such as food processing[2], pulp-paper industries and bioremediation[3].
To date, crystal structures of several laccases from Basidiomycetes have been determined, whereas only structures of three laccases from Ascomycetes are known.

What we know
Ascomycetes (belonging to the Dikarya kingdom) genomes encode a variety of laccases, but detailed biochemical and structural insight into these enzymes lags behind. The objective of this project is to investigate novel Ascomycete laccases, which are distinguished from previously characterized enzymes with respect to their catalytic modules and modular organization. These enzymes are encoded by plant pathogenic fungi, but their functional significance in pathogenesis remains unclear. We intend to study the biochemical and structural features of these enzymes to explore their potential as catalysts in biotechnological processes and to cast light on their biological roles in conjunction to fungal infection.

What we need
Plant researchers or mycologists with interest in investigating the biological role of laccases and other oxidative processes in fungal pathogenesis as well as scientists interested in applications of laccases and their electrochemical properties, e.g. in the biosensors area.

Acknowledgments
This project is funded by a personal COFUND Postdoc H.C Ørsted DTU grant to MHM (2014) and a grant from the Novo Nordisk Foundation within the area of "Biotechnology-based Synthesis and Production" for MAH.

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Mass spectrometry based identification and characterization studies of citrullinated protein biomarkers

Mandvi Sharma, Per Hagglund, Birte Svensson, Anne Christine Bay Jensen.

Brief description of research area

Citrullination or deamination is a post translational modification enzymatically catalyzed by a family of calcium dependent enzymes known as peptidylarginine deiminases (PADs). In mammals, the PAD family has five isoforms, PAD1-4 and 6. PAD enzyme activity is pH regulated (optimum at 7.4). Calcium concentration is the biggest regulator of PAD activity. Half of maximum PAD activity is obtained at a calcium concentration of 40-70μM and lower limit for a minimum PAD activity is observed around 10μM. All the PAD enzymes are seen in the cell cytoplasm, except PAD4 which has a nuclear localization. A prokaryotic PAD enzyme, having a sequence homology with deiminases has been identified in *Porphyromonas gingivalis* bacteria. Though this prokaryotic PAD enzyme does not show any evolutionary relationship to PADs from vertebrates, interestingly, its activity has been associated to rheumatoid arthritis. During citrullination the imine group of the guanidium group of arginine residues is substituted by ureido group, forming a non-standard amino acid citrulline. This substitution results in a molecular mass shift of 0.985 Da and loss of one positive charge per arginine residue modified, lowering the isoelectric point. Therefore, a change in charge distribution could result into protein unfolding, leading to degradation by proteolytic enzymes.

What we know

Citrullination plays a pronounced role in normal physiological events such as cell death. Nevertheless, this PTM also has a role in various autoimmune diseases and pathophysiologies such as cancer, multiple sclerosis and rheumatoid arthritis. Increase in citrullination is often detected in various inflammatory diseases; hence identification of citrullinated peptides from the autoantigens can be used as a biomarker for the diagnosis, prognosis and to monitor therapeutic treatment of these diseases.

What we need:

Citrullination will affect the biological activity of the proteins being modified. It will be quite interesting to investigate the changes as the exact relationship of citrullination to the disease and its progress is quite incomplete and meagre. Development of better investigation methods to solve this puzzle will be quite interesting.

Exploring complex glycan utilization machinery of *Roseburia* spp. implicated in inflammatory and metabolic disorders

Maria Louise Leth[1], Morten Ejby Hansen[1] and Maher Abou Hachem[1]
malole@bio.dtu.dk 1. Dept. of Systems Biology, Technical University of Denmark

The human gastrointestinal tract is colonized by a dense, dynamic and complex microbiota. Humans are unable to degrade most dietary glycans, which instead are metabolised by different gut bacteria in the lower part of the gastrointestinal tract. The metabolism of dietary complex polysaccharides by distinct gut microbiota taxa has a pronouced effect on human health and physiology, which is at least partly associated with the specific short chain fatty acid (SCFA) fermentation products of glycans that vary between different taxa.

To date, a relatively narrow taxonomic range of bacteria involving mainly probiotic strains from *Lactobacillus* and *Bfidobacterium* genera have been explored for their health promoting properties. The theraputic potential of other gut commensals remains ill-explored. Recent evidence correlates decreased abundance of gut commenal bacteria from the *Roseburia* genus to inflammatory and metabolic disorders such as Crohn‘s disease and type 2 diabetes (1-2). Furthermore, *Roseburia* spp. are reported to be primary degraders of complex polysaccharides including starch and inulin (3), which are fermented to butyrate. Butyrate production plays a role in the maintenance of colonic homostasis, and increased concentrations of this glycan fermentation product in the colon are associated with anti-inflammatory and anti-tumorgenic effects.

A more detailed investigation of the glycan metabolic reperotoire of *Roseburia* spp. is neccessary to reveal features underpinning its specialization and ultimately to explore its potential in therapeutic interventions targeting inflammatory and metabolic disorders. This project addresses the gap in our knowledge on the above aspects by characterization of selected glycan active proteins from the taxon. The latest results will be presented and discussed.

Acknowledgements
This study is supported by a Graduate School DTU Scholarship, Lyngby, Denmark

Literature

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2. Qin et al. 2012, *A metagenome-wide association study of gut microbiota in type 2 diabetes*, Nature, 490 (55–60).

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Barley limit dextrinase inhibitor as backbone for design of proteinaceous inhibitors of industrially important 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 the activity, but also stabilize their target enzymes. The presented project focuses on design of inhibitors of two groups of industrial enzymes, starch hydrolases and serine-proteases. Prolonging shelf-life and regulation of enzymes are important goals. Today chemicals are used, e.g. for reducing autoproteolysis of proteases. Inhibitors may also be used for recycling enzymes from industrial processes. A biochemically and structurally characterised limit dextrinase inhibitor (LDI)[1] will serve as template for computational protein design. LDI is from a family of α-amylase and serine-protease inhibitors, and we have evidence for interaction hotspots in the complex with its target enzyme. The combination of advanced computational methods and experimental characterisation of inhibitor variants offers a strong approach to achieve useful potent enzyme inhibitors.

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, and a Sapere Aude-Research Talent grant from the Danish Council for Independent Research|Technology and Production Sciences (to MSM).

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

Mass spectrometry-based structural characterization of cross-linked peptides and proteins

Michele Mariotti, Per Hägglund, Birte Svensson, Diana Julie Leeming

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 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.

One of my researches involve protein oxidation achieved by using Rose Bengal dye combined with visible light or by using AAPH as an intermediate for the generation of peroxyl radicals. These treatments would induce an alteration to selected proteins (lysozyme C, glucose 6-phosphate dehydrogenase and others); after investigation of SDS-PAGE gels of these proteins it is possible to see a dimer formation corresponding to a presumed cross-linking formation. The aim of my work is to elucidate the chemistry of these cross-links by using mass spectrometry techniques.

Other samples investigated in my research include human collagen type 3 pro-peptide, where a lot of cross-links are believed to be formed via transglutaminase and/or via lysyl oxidase. *Lactococcus lactis* thioredoxin reductase is another sample investigated in my research, where cross-linking formation is presumed to arise from the exposition of this enzyme to visible light.

What we know

The workflow used in my research consists of ^{18}O isotopic labeling combined with mass spectrometry analysis (MALDI-TOF and LC-MS/MS) with the aim of identifying unknown cross-links. I obtained positive results in terms of ^{18}O incorporation applied to some standard tryptic peptides after optimizing this strategy, and the same technique was used to achieve incorporation of ^{18}O in different cross-linked samples. From the data analysis of the oxidized samples I could pinpoint some cross-linked peptides where the cross-links are intramolecular and intermolecular dityrosine bonds. Elucidation of the chemistry of these cross-links has been done by manually investigating the MS/MS spectra of these selected peptides and different fragmentation modes have been compared (HCD, ETD and EtHCD). Some software such as Stavrox and SIMXL have also helped me in the cross-linking data analysis.

What we need

The workflow that I have established for the identification of cross-links without knowing with certainty their chemistry is only one approach for dealing with this issue, and it would be nice to have some alternative strategies. The software available for cross-linking identification for now are mostly concerning chemical cross-links and therefore not very helpful for my goal.

Proteogenomics in support of bi-specific chimeric antigen receptor target selection

Mike Bogetofte Barnkob[1], Christian Simon[2], Lars Rønn Olsen[1,3]
[1]MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom.
[2]Disease Systems Biology, Novo Nordisk Center for Protein Research, University of Copenhagen, Copenhagen, Denmark.
[3]Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark

Description of research area
Current clinical successes of chimeric antigen receptor (CAR) T cells have been achieved by targeting single, malignancy-specific surface molecules. In order to broaden the treatment applicability to more cancer types, progress is being made in developing bi-specific CARs that require binding to several ligands before lymphocytes are activated. Such technical advances hold the potential to increase the specificity of the therapy and thereby decrease off-target effects. Selecting good bi-specific CAR targets is balance between specificity and sensitivity of tumor cell targeting - High specificity is required for clinical safety, and high sensitivity is required for efficient clearance of the targeted cancer type.

Well-defined cell surface protein expression profiles are needed to facilitate target selection. Defining surface protein expression profiles for CAR therapy in silico require information about expression of a large number of surface proteins on a large number of cells at different states and differentiation stages. At present, no high-throughput technique for measuring expression of large numbers of surface proteins exists. However, surface molecule expression on individual cells has been measured at low rates using immunohistochemistry or flow cytometry for decades, and vast amounts of cell-specific expression has been published. This represents a rich, but unstructured source of data and information.

What we know
To facilitate the definition of unique surface molecule profiles, we collected and organized large amounts of protein expression data on human hematopoietic cells from the primary literature. To do so, we employed text mining techniques for article classification and subsequently extensive manual curation. We coupled this data with analysis of large-scale transcriptomics data for the surface proteins in order to assemble a data foundation for deep characterization of cell surface profiles. The resulting database contains expression of 305 surface proteins across 206 hematopoietic cells, totaling 9,153 data points. We then developed algorithms for data mining to define unique protein expression profiles for highly sensitive and specific CAR targeting. Ongoing efforts will expand the database to contain surface protein expression for cells in all human tissues, as well as experimental validation of potential CAR targets.

What we need
Although the collected data is likely to reflect reality in most instances, underlying information about co-expression is lost when collecting expression data on a protein-by-protein basis. For example, T cells can be both CD8+ and CD4+, but only at a very limited time in T cell development are they positive for both - the rest of the T cell life cycle they are positive for one or the other. We know this from the literature, but suspect that many more cases such as this exists for less characterized molecular markers. We are therefore looking to experimentally validate certain combinations of molecular markers on a number of cell lines, either by flow cytometry or mass cytometry.

Additionally, we are looking to expand the database beyond hematopoietic cells to comprise all cell types of the body (both healthy and malignant) and are looking for domain experts to contribute to this project by helping us define the cell types in different systems and tissues for which we will proceed to collect data.

Optimization of anti-cobratoxins for treatment of neurotoxic envenomings

Mireia Solà1, Andreas H. Laustsen2*, Lars Olsen2, Mads Clausen3, Brian Lohse2
1Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark
2Department of Drug Design and Pharmacology, University of Copenhagen, Denmark
3Department of Chemistry, Technical University of Denmark, Kgs. Lyngby, Denmark

Brief description of research area
Cobras (*Naja* spp.) are some of the most venomous and dangerous snakes worldwide, responsible for high mortality and morbidity. The most toxic components of cobra venoms are cobratoxins, which target the nicotinic acetylcholine receptors (nAChRs) responsible for neuromuscular transmission. Inhibition of nAChRs may lead to respiratory arrest with death as a result within 3-12 hr after a bite from a cobra. Early parental administration of appropriate antivenom is the cornerstone of life saving snakebite therapy. However, current antivenoms are still produced by animal immunization, which is a laborious and expensive process yielding highly immunogenic antivenoms due to the heterologous nature of equine antibodies in the antivenom. In contrast, novel antivenom based on synthetic peptides may offer an alternative solution, which is less expensive and cause less side effects.

What we know
A peptide-based antitoxin lead was previously discovered in our lab through phage display selection. The lead binds to α-cobratoxin in ITC experiments and is able to inhibit α-cobratoxin binding to the nAChR in TEVC experiments.

What we need
Currently, we are working in an approach for optimizing the peptide lead by designing and characterizing improved analogues. The poster will include the methodology and results of optimized peptide leads to include in promising peptide-based antitoxins.
This may pave the way for treating the clinical manifestations of neurotoxic envenomings caused by long α-neurotoxins, thus providing protection against many cobra species.

Conformational dynamics in the acidic loop of E2 ubiquitinating enzymes

Miriam Di Marco[1,2], Donald E. Spratt[3], Gary Shaw[3] and Elena Papaleo[1]

1. Computational Biology Laboratory, Unit of Statistics, Bioinformatics and Registry, Danish Cancer Society Research Center ,2100 Copenhagen , Denmark.
2. Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari *Aldo Moro* Via Orabona, 4 - 70125 Bari, Italy
3. Department of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada

Brief description of research area

E2-enzyme, Cdc34 and Ube2g2, are modulated by phosphorylation, are involved in ubiquitination pathway and are associated to cancer diseases.

I am studying conformational dynamics of E2 enzymes through different computational techniques (such as conventional Molecular Dynamics - NMR Chemical Shifts as replica-averaged restraints in MD - Well-tempered Metadynamics coupled to parallel tempering approaches). I am using these approaches to describe open and closed conformation in the unphosphorylated E2 enzyme, to study the accessibility of the catalytic cysteine and to evaluate effects of cancer-related mutations on opening/closing mechanism.

What we know

I am working at the newborn Computational Biology Laboratory as an Erasmus student. In our group, we have a plethora of different computational approaches to investigate structure-function relationship in key biological proteins. One of our main interests is to understand the effects of mutations and post-translational modifications on the native dynamics of the protein.

What we need

We are always looking for collaborators expert in biophysics and biochemistry with a focus on the key players in the ubiquitation pathway. The collaborations with this kind of groups would be extremely important for us to validate our predictions or to propose/design new experiments.

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

Exploiting homology information in non-template based prediction of protein structures

Paolo Marcatili[1]

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

Brief description of research area:
Even though the number of solved structures is steadily growing, there are a number of problems in which non-template based method for protein structure prediction are indispensible: prediction of proteins for which no homolog with a solved structure can be found (e.g. enzymes identified from metagenomic data), and loop structure prediction (e.g. antibody and TCR hypervariable loops).
By integrating sequence-derived information using a combination of physical, statistical and machine learning methods, we can significantly improve the accuracy of the available methods for *de novo* structure prediction.

What we know:
We have developed methods for the accurate predictions of lymphocyte receptors (antibodies and T cell receptors) in a complete automated way, and for the *de novo* modeling of proteins.

What we need:
Sequences of antibodies, T cell receptors, immunogenic proteins, or any proteins for which template-based models can not be produced.
Chemical cross-linking and mass-spectrometry data.

Transmission electron microscopy for morphology characterization of virus like particles

R.V. Mateiu[1], J. Thomsen[2], F.S. Pedersen[2], M. Duch[2], and J. Birkedal Wagner[1]

[1]DTU Cen, Center for Electron Nanoscopy, technical University of Denmark
[2]Department of Molecular Biology and Genetics, Aarhus University

Brief description of research area
Denmark produces about 4500 tons pig meat per day and about 90 % of this is exported. This results in an annual export of 4 milliards EUR and represents about 5% of the total income Denmark is making on all export. Porcine reproductive respiratory syndrome virus (PRRSV) causes about 43.6 million EUR losses per year in Denmark, and therefore controlling the virus is a top priority.
The commercially available PRRSV vaccines are not effective in the prevention and extermination of the virus and therefore new development routes, such as virus like particles (VLPs) are approached for obtaining efficient and safe vaccines, capable of addressing prevalent strains.
The project is a consortium between 8 partners from academia and industry (for details see www.pigvac.dk). Our role in the project is to apply electron microscopy for morphological characterization of VLPs.

What do we know
We know that negative staining and cryo transmission electron microscopy are the electron microscopy visualization options.

What we need
We have experience with advanced electron microscopy techniques for hard matter. The soft matter part, which includes organic and biological materials, is currently under development and therefore would like to meet other people currently using electron microscopy for visualization and characterization of viruses and proteins.

Implementaion of online electrochemical reduction of proteins into a H/D exchange mass spectrometry workflow

Rasmus U. Jakobsen 1, Esben Trabjerg 1,2, Simon Mysling 3, Søren Christensen 2, Thomas J. D. Jørgensen 3, and Kasper D. Rand 1
1Department of Pharmacy, University of Copenhagen, Copenhagen E, Denmark, 2Department of Biologics, H. Lundbeck A/S, Valby, Denmark, 3Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

Brief description of research area
Until now heavily disulfide-bonded regions could present a formidable, sometimes even insurmountable resistance towards reduction in a Hydrogen/Deuterium exchange mass spectrometry (HDX-MS) workflow. This is due to the need for reduction of disulfide bonds prior to proteolytic digestion. Previously electrochemical reduction has shown promising results as to implementation into the HDX-MS workflow. Here, we show the successful implementation of electrochemical reduction into an HDX-MS workflow.

What we know
We know how to implement the cell into the HDX workflow and have done some optimizations on working with this.

What we need
We need want to know the effect of denaturing agents eg. Guadine HCl and Ureas effect on performance of the cell.

Developing of microbial consortia for enzymatic valuable conversion of keratin-rich slaughter-house waste

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. Instead of using single organisms, an attempt will be made to investigate the relationship between microorganisms in consortia and their combined ability to degrade keratin. Growing microorganisms in media that enrich for keratin degrading organisms, consortia that use milled pig bristles as their sole carbon and nitrogen source have been produced. Using mass spectrometry and biochemistry based investigation techniques, the methods by which these organism 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. Furthermore, the possible interactions and symbiosis between microorganisms in a consortia will be investigated on a proteomics level. The hope is that this will not only further the understanding of the degradation of keratin rich fibers, but also attribute with knowledge about the interactions between microorganisms.

Preparation and self-assembly of amphiphilic polylysine dendrons

Sahar Mirsharghi[1], Kenneth D. Knudsen[2], Shahla Bagherifam[3,4], Bo Nyström[3], Peter M. H. Heegaard[1] and Ulrik Boas[1].

[1] DTU Vet, Section for Immunology and Vaccinology, Copenhagen, Denmark.
[2] Institute for Energy Technology, Department of Physics, Kjeller, Norway.
[3] University of Oslo, Department of Chemistry, Oslo, Norway.
[4] University of Oslo, Department of Biology, Oslo, Norway.

Brief description of research area
We work with solid-phase synthesis of well-defined and hyperbranched polylysine molecules (dendrons) by a method developed in our research group. In the method an aldehyde linker is employed in the first synthetic step. This allows for the introduction of a C-terminal functional group which otherwise would not be possible. Hereafter, the polylysine core is built up by normal divergent peptide coupling chemistry. The N-terminal groups of the free amino groups of the lysines can further be modified with molecular motifs of interest resulting in a multivalent display. We currently work on the synthesis of these dendritic molecules with immunomodulatory motifs covalently linked to the dendritic surface. Our aim is to elucidate the effect of multivalent display of immunomodulatory motifs on immune cells.

What we know
A small library of amphiphilic polylysine dendrons were synthesized with varying alkyl chain lengths at the C-terminal without N-terminal immunomodulatory motifs. Physicochemical studies (small angle neutron scattering and dynamic light scattering) were performed on these dendritic molecules.

Small angle neutron scattering (SANS) revealed self-assembly of these amphiphilic dendrons into micellar structures under aqueous conditions. Only dendrons with an alkyl chain length above C12 had self-assembling properties. The SANS data were fitted by a core-shell model with the lipophilic alkyl chain forming the core and the hydrophilic lysine groups forming the shell/surface. The micellar diameters obtained by SANS were approximately 6 nm and fitted well with two dendrons spanning the diameter.

Dynamic light scattering (DLS) showed large intermicellar aggregates (65-370 nm) by mechanisms not directly related to the conventional hydrogen and hydrophobic interactions. Increasing the temperature and concentration did not have any significant effect on the hydrodynamic sizes obtained by DLS. However, when examining different pH values it was clear that an acidic pH was critical for intermicellar aggregation. This suggested the need for positively charged amines on the lysine surface groups. Hence, electrostatic forces seemed to be crucial for intermicellar aggregations.

Additionally, we observed by SANS that partial removal of positively charged amine groups by acetylation had a stabilizing effect on the micelle forming ability. This suggested a more complex relationship between surface charge and aggregation behavior.

The cytotoxicity of the amphiphilic dendrons was evaluated in mouse fibroblasts (NIH/3T3) and human embryonic kidney (HEK 293T) cells at 5, 10, 20 μM concentrations. Contrary to the expected cytotoxicity profile due to positively charged amine groups, the dendrons proved to be biocompatible with cell viabilities well above 80 % for all compounds. This indicates the potential for further development of these dendritic molecules for biomedical applications.

What we need

- **Molecular simulations:**
  We want to elucidate the mechanisms behind the formation of micelles and larger intermicellar aggregates.

- **cryoTEM:**
  To obtain a better picture of how the micelles and intermicellar structures look.

- **Immunomodulation:**
  We are interested in new chemical ligands or immunologically relevant molecular motifs for covalent coupling to our dendrons to investigate the effect of multimericity on their biological properties.

- **High resolution mass spectrometry:**
  Tips for setting up high resolution MS on our ESI-TOF system (Bruker) is highly appreciated, since we would like to include this analysis on the novel dendritic molecules.

Interaction studies of bacterial exopolysaccharides with β-lactoglobulin by solution X-ray scattering and dynamic light scattering

Sanaullah Khan[1,2], 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 the interactions of bacterial exopolysaccharides with β-lactoglobulin in solution.

What we know
To study the interactions between EPS and β-lactoglobulin, we performed analytical ultracentrifugation (AUC), dynamic light scattering (DLS) and X-ray solution scattering with bacterial EPSs and β-lactoglobulin. AUC sedimentation velocity data for β-lactoglobulin and its mixtures with EPS-GG gave sedimentation coefficients of 2.7 S and ~29 S respectively. 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 bacterial EPSs increased significantly from 2.3 to 3.1 nm, and the maximum lengths of β-lactoglobulin increased from 7.5 to 10.5 nm, confirming that large particles had formed.

What we need
To study the morphology of β-lactoglobulin-EPS particles, and to gain insight into how β-lactoglobulin binds to EPS, cryo-electron microscopy (cryoEM) would be useful to use.

MD-Coevol refinements of homology models – GromacsWrapper

Sanne Bergstrand-Poulsen[1,2] , Peter Røgen [2] and Elena Papaleo [1]

1 Computational Biology Laboratory, Unit of Statistics, Bioinformatics and Registry, Danish Cancer Society Research Center ,2100 Copenhagen , Denmark.
2 2. DTU COMPUTE, Technical University of Denmark**,** Richard Petersens plads, Building 324, DK-2800 Kgs. Lyngby, Denmark

Brief description of research area
Improving the quality of protein comparative modeling with low sequence identity by integrating coevolution information and molecular simulations.

What we know
My main field of interest is how to apply mathematical modelling to biological systems and data. In the research group at DCRC where I'm currently doing my thesis molecular modelling of protein structures and state of the art analysis of high-throughput data are some of the focus areas.

What we need
Input from researchers in molecular modelling and neighbouring fields in order to validate and delevop our output would be very beneficial as well as improve my knowledge of what other groups are working on.

N-terminal selective introduction of azides in proteins by pH-controlled diazotransfer

Sanne Schoffelen[1], Surendra Vutti[1], Hongxia Hu[1], Morten Meldal[1]

Center for Evolutionary Chemical Biology, Department of Chemistry, University of Copenhagen
Universitetsparken 5, 2100 Copenhagen, Denmark, e-mail: sanne@chem.ku.dk

Brief description of the research area
The Cu(I)-catalyzed alkyne–azide cycloaddition (CuAAC) and its copper-free, strain-promoted variant (SPAAC) are highly efficient and popular reactions for protein conjugation. In order to facilitate the use of these so-called 'click' reactions, several methods have been developed to introduce azides and alkynes in proteins. Among these methods are enzyme-mediated strategies, non-natural amino acid incorporation and intein-mediated protein ligation. The chemical conversion of amines into azides *via* an aqueous diazotransfer reaction is an attractive alternative, as it does not require any genetic engineering.

What we know
Using metal-free and pH-controlled conditions, the diazotransfer reaction was shown to be selective towards the amine with the lowest pK_a, that is, the α-amine at the N-terminus of tripeptide Lys-Phe-Phe, and proteins *Candida antarctica* lipase B and elastin-like polypeptide. More recently, we demonstrated that the reaction is also highly specific towards the N-terminus of green fluorescent protein (GFP), probably the most widely used model in protein conjugation studies. GFP was immobilized on polymeric beads and semiconductor nanowires, both acting as potential platform for protein–protein interaction assays.

What we need
A better understanding of the mechanism underlying the site-selectivity of the diazotransfer reaction will allow us to assess the potential limitations and applicability of the reaction as protein modification strategy.

Characterization of a GH62 α-L-arabinofuranosidase from *Aspergillus nidulans*: Linking functional diversity with phylogenetics

Susan Andersen[1], Casper Wilkens[1,2], Bent O. Petersen[3], Barry McCleary[4], Ole Hindsgaul[3], Maher Abou Hachem[1] and Birte Svensson[1]

1. Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark;
2. Department of Chemical and Biochemical Engineering, Technical University of Denmark, Kgs. Lyngby, Denmark; 3. Carbohydrate Chemistry Group, Carlsberg Laboratory, Valby, Denmark; 4. Megazyme International, Bray, Co. Wicklow, Ireland

Brief description of research area

L-arabinofuranose (Ara*f*) residues are abundant in plant cell walls as L-arabinan chains of pectin and side chains in arabinoxylan, arabinogalactan and gum arabic. Removal of Ara*f* residues is important in complete degradation of plant material in various industrial processes such as bioethanol production (Jordan *et al.*, 2012). The present *Aspergillus nidulans* L-arabinofuranosidase (AnAbf62A-m2,3) of glycoside hydrolase family 62 (GH62) displays unusually high activity for low viscosity wheat arabinoxylan and moderate ability to hydrolyse sugar beet L-arabinan and 4-nitrophenyl-α-L-arabinofuranoside. NMR studies revealed that AnAbf62A-m2,3 hydrolyses Ara*f* from singly α-1,2 and α-1,3-substituted xylose residues releasing α-1,3-Ara*f* three times faster than α-1,2-Ara*f*. It has been shown that arabinoxylans that contain singly α-1,2 and α-1,3-Ara*f* have to be oriented oppositely for hydrolysis (Wang *et al.*, 2014). The slower rate of α-1,2-Ara*f* release may thus reflect that arabinoxylan binds weaker in the orientation that is compatible with hydrolysis of α-1,2-Ara*f* from xylose of the backbone. NMR analysis further showed that AnAbf62A-m2,3 releases product with inverted anomeric configuration, as for the GH43 (Pitson *et al.*, 1996), the other clan GH-F member (Lombard *et al.*, 2014).

Finally, phylogeny and analysis with the Peptide Pattern Recognition (PPR) tool revealed that sequences within the GH62 are divide into tree distinctive subfamilies, which differ from the two subfamilies, which the GH62 family was previously divided into (Siguier, 2014). The PPR analysis further revealed sequence motifs that distinguish the three subfamilies, which might be important for the functionality of the GH62s within each subfamily.

What we know

- AnAbf62A-m2,3 displays high activity for low viscosity wheat arabinoxylan and moderate ability to hydrolyse sugar beet L-arabinan and 4-nitrophenyl-α-L-arabinofuranoside.
- NMR studies revealed that AnAbf62A-m2,3 hydrolyses Ara*f* from singly α-1,2 and α-1,3-substituted xylose residues releasing α-1,3-Ara*f* three times faster than α-1,2-Ara*f*. It was also shown that AnAbf62A-m2,3 releases product with inverted anomeric configuration.
- The PPR analysis revealed sequence motifs that might be important for the functionality of the GH62s within each subfamily.

What we need

- Arabinoxylan and arabinan oligosaccharides

Convolutional LSTM networks for subcellular localization of proteins

Søren Kaae Sønderby[1], Casper Kaae Sønderby[1], Henrik Nielsen[2], and Ole Winther[1,3].
[1]Bioinformatics Centre, Department of Biology, University of Copenhagen.
[2]Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark.
[3]Department for Applied Mathematics and Computer Science, Technical University of Denmark.

Brief description of research area
Predicting the subcellular locations of proteins from their amino acid sequences constitutes an important task in bioinformatics, and many methods have been published. The methods can be divided into those that use only the sequence for prediction, and those that search databases for metadata which are then used in the prediction. Machine learning is widely in this area. Non-sequential models such as SVMs or feed-forward neural networks are often used although they have no natural way of handling sequences of varying length. Recurrent neural networks such as the long short term memory (LSTM) model on the other hand are designed to handle sequences.

What we know
In this study we demonstrate that LSTM networks predict the subcellular location of proteins given only the protein sequence with high accuracy (0.902) outperforming current state of the art algorithms. We further improve the performance by introducing convolutional filters and experiment with an attention mechanism which lets the LSTM focus on specific parts of the protein. Lastly we introduce new visualizations of both the convolutional filters and the attention mechanisms and show how they can be used to extract biologically relevant knowledge from the LSTM networks.

What we need
We plan to retrain our convolutional LSTM network model on new data derived from the current version of UniProt. However, since UniProt approximately one year ago changed their “evidence ontology” and decided to mark only those annotations as “experimental” where a relevant literature reference could be found, there are now far fewer proteins in UniProt with subcellular location regarded as experimentally confirmed. We are therefore searching for additional sources of high-quality experimental annotations of subcellular locations of proteins as well as exploring possibilities for utilising the vast amounts of unlabeled protein data.

Prevalence of *N*-acetyl hexosaminidases and α-fucosidases in bacteria isolated from cold environments

Triinu Visnapuu[1], Peter Stougaard[2], Morten Jepsen[2], Bettina Nonneman[3], Branko Kokotovic[3] 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]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark
[3]National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark

β-*N*-acetyl hexosaminidases (EC 3.2.1.52) and α-L-fucosidases are (EC 3.2.1.51) are considered as enzymes with biotechnological interest due to their ability to produce bioactive and potentially prebiotic oligosaccharides. These enzymes are widespread in nature and are involved in important biological processes catalyzing the cleavage of terminal *N*-acetyl hexosaminyl and α-L-fucosyl moieties, respectively, from different types of glycosaminoglycans and glycoconjugates. The prevalence and diversity of bacterial species with above-mentioned activities in different environments, *i.e.* cold environments have not been addressed before. Screening of bacterial species from various biotopes for the activities could give rise to novel β-*N*-acetyl hexosaminidase and α-L-fucosidase variants which could be used as tools in biotechnology.

Techniques used in the study involve isolation of bacterial species, culture-dependent screening methods for β-*N*-acetyl hexosaminidase and α-L-fucosidase activities and spectrophotometric quantification of specific enzymatic activities. The bacterial species were identified using high-throughput MALDI-TOF MS-based method coupled with 16S rRNA gene sequencing.

Additionally, the current work will benefit from expertise and techniques involving specific oligosaccharide detection and quantification methods and solvent-aided transglycosylation.

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

Enzymatic preparation of linear isomaltomegalo-saccharide by *Gluconobacter oxydans* dextran dextrinase

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

[1]DTU Systems Biology. [2]Research Faculty of Agriculture, Hokkaido University.

Brief description of research area

The megalosaccharide has DP between 10 and 100. Linear isomaltomegalosaccharide (L-IMS), which is linear-type α-(1→6)-glucosyl megalosaccharide, possesses 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.

Decoding Nitrosylation in Bacteria and Humans

Zacharias Brimnes Visby Damholt*1*, Susanne Jacobsen*1*, Anne-Christine Bay-Jensen3, Birte Svensson*2* and Per Hägglund*1*

1Protein and Immune Systems Biology 2Enzyme and Protein Chemistry 3Nordic Bioscience

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.

What we know:
Mass spectrometry based proteomics, Q-Exactive. Data interpretation of mass spec results. Analysis of post-translational modifications. Investigating novel and unknown interaction partners to proteins.

What we need:
In vitro system for nitration and nitrosylation of proteins and peptides. Patient samples

MD Calculation of the stability and binding to opioid receptor of D/L-Dermorphin

Author: NN

The ability to identify high affinity an agonist between potential binders is of utmost importance in the understanding and design of ligands. Applications using peptides are increasing and to alter their stability it is necessary to modify them. It is important that the modification does not change the bioactive conformation of the peptide, which can decrease their function.

Dermorphin is a neuropeptide, which has a high affinity and selectivity to the μ-receptor and induces a strong signal. The peptide is modified from nature andcontains a D-amino acid at the second position. This relatively small substitution increases its binding constant 10.000 times compared with the pure L-isomeric

form. Here we simulate the two isomers to investigate their differences using explicit molecular dynamics simulation and replica exchange in implicit solvent. The trajectories show that the D-isomeric replacement makes the natural occurring peptide more restricted in the dihedral geometry around the substitution as well as its backbone. The decreased flexibility in conformational space correlates well with experimental results.

This study indicates that strong agonists do not fight against conformational entropy and could be used as a filter to test modified peptides.

List of Participants

First name	Surname	Title	Affiliation	E-mail	Poster no
Abida	Sultan	Postdoc	DTU Biosustain	absu@biosustain.dtu.dk	
Agnieszka	Z. Nielsen	Postdoc	KU-PLEN	az@plen.ku.dk	1
1Ali	Fallah	PhD	Novozymes	aifa@novozymes.com	
Allan	Svendsen	Science Director	Novozymes	asv@novozymes.com	
Amelie	Stein	PhD	University of Copenhagen	ameliestein25@gmail.com	
Ana Rita	Colaço	PhD Student	DTU Chemistry	anaco@kemi.dtu.dk	3
Anna	Leuchsenring	PhD Student	DTU Vet	ableu@vet.dtu.dk	
Anna Bielecka	Henriksen		Statens Serum Institut	xjz561@alumni.ku.dk	2
Anne Veller Friis	Nielsen	PhD	DTU Chemical Engineering	avfn@kt.dtu.dk	
Annemarie	Matthes	Dr	CPSC University of Copenhagen	annemarie.matthes@plen.ku.dk	
Antoni	Malagocki	Student	University of Copenhagen	hsx490@alumni.ku.dk	
Artur	Wlodarczyk	PhD Student	University of Copenhagen	ajw@plen.ku.dk	
Arun Kumar	Somavarapu	Mr	DTU Chemistry	arkuma@kemi.dtu.dk	
Avishek	Majumder	Mr	Einar Willumsen	ama@ewflavours.com	
Bao-Wen	Yuan	Master Student	DTU Systems Biology	yuanbaowen521@126.com	
Birgitte	Zeuner	Postdoc	DTU Chemical Engineering	biz@kt.dtu.dk	
Bjarne	Ersbøll	Professor	DTU Compute	bker@dtu.dk	

Bjørg	Schulz	Ms	University of Copenhagen	Bjn938@alumni.ku.dk	
Bo	Pilgaard	AC-TAP	DTU Chemical Engineering	bpil@kt.dtu.dk	
Brian	King	PhD	Cornell University	bck22@cornell.edu	4
Budi Juliman	Hidayat	Fermentation Manager	UniBio A/S	bjh@unibio.dk	
Catharina	Stenholm	Manager	Novozymes	ctst@novozymes.com	
Carsten	Andersen	Mr	Novozymes	cara@novozymes.com	
Casper	Wilkens	Postdoc	DTU Chemical Engineering	cwil@kt.dtu.dk	
Casper	Højgaard	PhD Student	University of Copenhagen	casperhoejgaardmail@gmail.com	
Cecilia	Emanuelsson	Professor	Lund University, Sweden	Cecilia.Emanuelsson@biochemistry.lu.se	5
Chris Juul	Hedegaard	Postdoc	DTU Vet	cjhe@vet.dtu.dk	
Christian	Vestergaard	Manager	CO-RO A/S	csvestergaard@co-ro.com	
Christian	Kjeldsen	PhD Student	DTU Chemistry	chkje@kemi.dtu.dk	
Christian	Kofoed	PhD Student	University of Copenhagen	christian.kofoed@chem.ku.dk	
Christine	Finnie	Senior Scientist	Carlsberg Laboratory	Christine.Finnie@carlsberglab.dk	
Claus	Felby	Professor	University of Copenhagen	cf@ign.ku.dk	
Comamala	Gerard		University of Copenhagen	gerardkoma@gmail.com	9
Cyrielle	Calmels	PhD Student	DTU Systems Biology	cyrielle.calmels@ucb.com	
David	Cannella	Postdoc	University of Copenhagen	dac@ign.ku.dk	

Dolores	Vicovic	MsC		doloresvictovic@gmail.com	
Elena	Papaleo	Group Leader	Danish Cancer Society Research Center	elenap@cancer.dk	
Emil	Dedic		Statens Serum Institut		6
Emil G. P.	Stender	PhD Student	DTU Systems Biology	emigst@bio.dtu.dk	7
Fabio	Doro	Dr	University of Copenhagen	fabio.doro@bio.ku.dk	
Folmer	Fredslund	Dr	Department of Chemistry, University of Copenhagen	folmer@chem.ku.dk	8
Frank	Rasmussen	Science Manager	Novozymes	Fwr@novozymes.com	
Giuseppe	Dionisio	PhD	Aarhus University	giuseppe.dionisio@agrsci.dk	10
Gleb	Dotsenko	Postdoc	DTU Chemical Engineering	gdot@kt.dtu.dk	
Hengtao	Wang	Master Student	DTU Systems Biology	wanght@big.ac.cn	
Henrik	Nielsen	Associate Professor	DTU Systems Biology	hnielsen@cbs.dtu.dk	
Henrik	Bohr	Dr	DTU Physics	hbohr@fysik.dtu.dk	
Henrik	Stålbrand	Professor	Lund University, Sweden	henrik.stalbrand@biochemistry.lu.se	
Hoff	Tine	Science Manager	Novozymes	thff@novozymes.com	
Hongxia	Hu	PhD Student	Center for Evolutionary Chemical Biology	hongxia.hu@chem.ku.dk	
Husam	Alsarraf	Mr	Statens Serum Institut	hual@ssi.dk	11
Ingemar	Andre	Associate Professor	Lund University, Sweden	ingemar.andre@biochemistry.lu.se	12
Jakob	Bohr	Professor	DTU Nanotech	jabo@nanotech.dtu.dk	

Javier	Linares-Pastén	Dr	Lund University, Sweden	javier.linares_pasten@biotek.lu.se	
Jens	Vogensen	PhD Student	DTU Systems Biology	jenvo@bio.dtu.dk	13
Jeppe	Tams	Science Manager	Novozymes	jwta@novozymes.com	
Jeppe	Mouritsen	Researh Scientist	Novozymes	jchm@novozymes.com	
Jesper	Holck	Researcher	DTU Chemical Engineering	jeh@kt.dtu.dk	
Jianming	Liu	Mr	DTU Food	jial@food.dtu.dk	
Jinxia	Xu	Master Student	DTU Systems Biology	1029701890@qq.com	
João	Martins	Mr	University of Copenhagen, SbinLab	joao.martins@bio.ku.dk	
Johanne	Hadsbjerg		DTU Vet	johad@vet.dtu.dk	
Johnny	Birch	PhD Student	DTU Systems Biology	birch@bio.dtu.dk	14
Jose	Izarzugaza	Associate Professor	DTU Systems Biology	txema@cbs.dtu.dk	
Kaj Frank	Jensen	Professor Emeritus	Department of Biology, University of Copenhagen	kajfrankjensen@gmail.com	
Karina	Jansen	Technician	DTU Systems Biology	kara@bio.dtu.dk	
Kasper	Tidemand	PhD Student	DTU Chemistry	kdati@kemi.dtu.dk	
Kasper	Rand	Associate Professor	University of Copenhagen	kasper.rand@sund.ku.dk	
Kasper	Enemark-Rasmussen	AC-TAP	DTU Chemistry	keras@kemi.dtu.dk	
Kenny	Ståhl	Assistant Professor	DTU Chemistry	kenny@kemi.dtu.dk	
Kresten	Lindorff-Larsen	Assistant Professor	University of Copenhagen	lindorff@bio.ku.dk	

Kristian	Davidsen	Student	DTU Systems Biology	krdav@cbs.dtu.dk	
Kristoffer	Laugesen	Mr	BioNanotechnology and NanoMedicine Lab, UCPH	bfs190@alumni.ku.dk	
Lars Rønn	Olsen	Assistant Professor	DTU Systems Biology	lro@cbs.dtu.dk	
Laura Rokkedahl	Grauslund	Ms	Copenhagen University, Denmark	Rokkedahlgrauslund@gmail.com	
Lene Holberg	Blicher	Lab Manager	DTU Systems Biology	lench@bio.dtu.dk	
Leonardo	De Maria	Principal Scientist	Novo Nordisk	LeDM@novonordisk.com	
Line	Lauritsen	Bachelor Student	University of Copenhagen	klp501@alumni.ku.dk	
Line Abildgaard	Ryberg	Miss	DTU	linearyberg@gmail.com	
Linette	Buxbom	Molecular Biologist	DTU Vet	linbubo@vet.dtu.dk	
Majid	Haddad momeni	Postdoc	DTU Systems Biology	mahad@bio.dtu.dk	15
Mandvi	Sharma	Mrs	DTU Systems Biology	mansh@bio.dtu.dk	16
Maria	Blanner Bang	Chemist	DTU Chemistry	mabl@kemi.dtu.dk	
Maria Louise	Leth	PhD Student	DTU Systems Biology	malole@bio.dtu.dk	17
Maria Maddalena	Sperotto	PhD	DTU Systems Biology	maria@cbs.dtu.dk	
Marie Sofie	Møller	Postdoc	DTU / Lund University	mariesofiemoller@gmail.com	18
Martin	Rykær	Mr	DTU Systems Biology and DTU Biosustain	marry@bio.dtu.dk	
Micha Ben Achim	Kunze	Dr	University of Copenhagen	mbakunze@gmail.com	

Michele	Mariotti	PhD Student	DTU Systems Biology	micmar@bio.dtu.dk	19
Mike	Barnkob		Copenhagen University		20
Mireia	Solà	Researcher	Department of Drug Design and Pharmacology, University of Copenhagen	mireiasc.9@gmail.com	21
Miriam	Di Marco	Ms	Danish Cancer Society Research Center	mirod22@hotmail.it	22
Mustapha Carab	Hussein	Master Student	University of Copenhagen, SBiNLab	gmt259@alumni.ku.dk	
Nanda Gowtham	Aduri	Mr	University of Copenhagen	nanda.aduri@sund.ku.dk	
Nicklas	Skjoldager	PhD Student	DTU Systems Biology	nics@bio.dtu.dk	23
Nicklas Patrick	Sørensen	Master Student	DTU Systems Biology	nicklas.p.sorensen@gmail.com	
Nicolai	Tidemand Johansen	Mr	University of Copenhagen	Mct829@ku.dk	
Paolo	Marcatili	Mr	DTU	paolo.marcatili@gmail.com	24
Parvin	Shakeri Fard	Postdoc	DTU Food	parsh@food.dtu.dk	
Peng	Wu	Researcher	DTU Chemistry	penwu@kemi.dtu.dk	
Per	Hägglund	Associate professor	DTU Systems Biology	ph@bio.dtu.dk	
Per	Greisen	Dr	Novo Nordisk	pjug@novonordisk.com	
Pernille	Sønderby	PhD Student	DTU Chemistry	perso@kemi.dtu.dk	
Peter	Fristrup	Associate Professor	DTU Chemistry	pf@kemi.dtu.dk	
Peter	Røgen	Associate Professor	DTU Compute	prog@dtu.dk	

Philipp	Gruner	Dr	Novozymes	ppg@novozymes.com	
Qianqian	Sun	Master Student	DTU Systems Biology (Sino-Danish College of Chinese Academy of Science)	sunqian124@126.com	
Qijin	Chi	Associate professor	DTU Chemistry	cq@kemi.dtu.dk	
Radhakrishna	Shetty	Dr	DTU Food	rads@food.dtu.dk	
Ramona	Mateiu	Researcher	DTU Center for Elektron Nanoskopi	ramona.mateiu@cen.dtu.dk	25
Rasmus	Jakobsen	Mr	Protein Analysis Group, Department of Pharmacy, University of Copenhagen	rujakobsen@gmail.com	26
René	Jørgensen		Statens Serum Institut	renj@ssi.dk	
Richard	Ipsen	Professor	Department of Food Science, University of Copenhagen	ri@food.ku.dk	
Roall	Espersen	PhD Student	DTU Systems Biology	roes@bio.dtu.dk	27
Roland	Pache	Dr	Novozymes	ROAP@novozymes.com	
Rosa	Jersie-Christensen	PhD Student	KU NNF CPR	rosa.rjc@cpr.ku.dk	
Ruth	Hendus-Altenburger	PhD	University of Copenhagen	rha@bio.ku.dk	
Sahar	Mirsharghi	M.Sc.	DTU Vet	sahmi@vet.dtu.dk	28
Saif	Ullah	Postdoc	DTU Nanotech	saul@nanotech.dtu.dk	
Salome	Veshaguri	PhD	University of Copenhagen	salome.veshaguri@nano.ku.dk	
Sanaullah	Khan	Mr	DTU Nanotech	sank@nanotech.dtu.dk	29

Sanne	Bergstrand	Master Student	DTU Compute and The Danish Cancer Society	sbp@cancer.dk	30
Sanne	Schoffelen	PhD	Chemistry Institute, Copenhagen University	sanne@chem.ku.dk	31
Sebastian	Meier	Senior Scientist	DTU Chemistry	semei@kemi.dtu.dk	
Shazia	Rehman	Ms	Copenhagen University, Denmark	shre@plen.ku.dk	
Shi	Huang	PhD	Department of Chemical Engineering	huangshi@qibebt.ac.cn	
Shiwen	Zhuang	Dr	DTU Food	shizh@food.dtu.dk	
Simon	Mysling	Postdoc	Finsen Laboratory	simon.mysling@finsenlab.dk	
Sindrila	Dutta Banik	Miss	DTU Chemistry	siduba@kemi.dtu.dk	
Sita	Vaag Olesen	PhD Student	DTU Systems Biology	sitavaagolesen@gmail.com	
Susan	Andersen	PhD Student	DTU Systems Biology	suj@bio.dtu.dk	32
Søren	Midtgaard	Postdoc	Copenhagen University, Denmark	soromi@nbi.ku.dk	
Søren Kaae	Sønderby		Copenhagen University, Denmark		33
Tem	Dang	PhD Student	University of Copenhagen	temthidang@food.ku.dk	
Thea	Kristensen		DTU Vet	thkri@vet.dtu.dk	
Thomas	Kledal	Head of Section	DTU Vet	tnkl@vet.dtu.dk	
Tine	Frederiksen	PhD Student	DTU Chemistry	timf@kemi.dtu.dk	
Tone	Bengtsen	Student	University of Copenhagen, SbinLab	tone_bengtsen@hotmail.com	
Triinu	Visnapuu	Dr	DTU Systems Biology	trivi@bio.dtu.dk	34
Tune	Wulff	Proteomic scientist	DTU Biosustain	tuwu@biosustain.dtu.dk	

Ulrik	Boas	Associate Professor	DTU Vet	uboa@vet.dtu.dk	
Verena	Volf	Master Student	DTU Systems Biology	vevol@bio.dtu.dk	
Vibeke	Orlien	Associate professor	University of Copenhagen	vor@food.ku.dk	
Willy	Bjørklund	Area Manager LSMS Denmark	Thermo Fisher Scientific	Willy.bjorklund@thermofisher.com	
Wojciech	Potrzebowski	Dr	Lund University, Sweden	wojciech.potrzebowski@biochemistry.lu.se	
Xianliang	Zheng	PhD	Department of Chemical and Biochemical Engineering	zhengxl@qibebt.ac.cn	
Xiaojing	Li	Master Student	DTU Systems Biology	lixj_bio@163.com	
Xunong	Dong	Master Student	DTU Systems Biology	916116833@qq.com	
Yuhong	Huang	Dr	DTU Chemical Engineering	yhua@kt.dtu.dk	
Yuya	Kumagai	Postdoc	DTU Systems Biology	yuyaku@bio.dtu.dk	35
Zacharias	Damholt	PhD Student	DTU Systems Biology	zada@bio.dtu.dk	36
NN	NN				37

Speakers

First name	Surname	Title	Affiliation	E-mail
Andreas	Dietl	PhD Student	Max Planck Insitute for Medical Research	Andreas.Dietl@mpimf-heidelberg.mpg.de
Annalisa	Pastore	Professor	King's College London	annalisa.1.pastore@kcl.ac.uk
Carme	Rovira	ICREA Research Professor	Universitat de Barcelona, Spain	c.rovira@ub.edu
Gunnar	von Heijne	Professor	Stockholm University	gvheijne@me.com
Julia	Chamot-Rooke	Directeur de Recherche	CNRS, Paris, France	julia.chamot-rooke@pasteur.fr
Kamrul	Hasan	PhD Student	Lund University, Sweden	Kamrul.Hasan@biochemistry.lu.se
Pier Giorgio	Righetti	Professor	Polytechnic of Milan	piergiorgio.righetti@polimi.it
Sarel	Fleishman	Assistant Professor	Weizmann Institute of Science, Israel	sarel.fleishman@weizmann.ac.il
Sebastian	Deindl	Assistant Professor	Uppsala University, Sweden	sebastian.deindl@icm.uu.se
Thomas	Vorup-Jensen	Professor	Department of Biomedicine, Aarhus University	vorup-jensen@biomed.au.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