Celebrating the First Decade

19th Workshop in

21 October 2019

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19th Workshop in Protein.DTU

Contents

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October 21, 2019

Building 101, Lecture Hall Glassalen

08:30 – 09:00 **Arrival & Breakfast**

09:00 – 09:10 **Welcome**
Bjarke Bak Christensen, Head of Department, DTU Bioengineering
Chair: Birte Svensson, Professor, DTU Bioengineering

09:10 – 09:40 ***Recombinant and Engineered Lectins for Research and Diagnostics***
Anne Imberty, Research Director, Centre de Recherches sur les Macromolécules Végétales (CERMAV), Grenoble, France
Chair: Anne S. Meyer, Professor, DTU Bioengineering

09:40 – 10:10 ***Mass Spectrometry Approaches to Dynamic Protein Structure: from Disorder to Membrane Pores***
Frank Sobott, Professor, The Astbury Centre for Structural Molecular Biology, University of Leeds, United Kingdom
Chair: Preben Morth, Professor, DTU Bioengineering

10:10 – 10:40 ***Solid-state NMR Based Structural Biology***
Antoine Loquet, Group Leader, Institut Européen de Chimie et Biologie (IECB) Université de Bordeaux, France
Chair: Alexander Buell, Professor, DTU Bioengineering

10:40 – 11:05 **Coffee Break**

11:05 – 11:35 ***Uncovering Protein Function Through Surface Analysis***
Emma Master, Professor, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Canada
Chair: Birte Svensson, Professor, DTU Bioengineering

11:35 – 12:05 ***Watching Molecules at Work: Protein Structural Dynamics Revealed by Femtosecond Crystallography***

Thomas Barends, Group Leader, Department of Biomolecular Mechanisms, Max Planck Institute for Medical Research, Heidelberg, Germany
Chair: Peter Heegaard, Professor, DTU Bioengineering

12:05 – 12:45 **Lunch and poster viewing**

12:45 – 13:30 **Poster Session, coffee/tea, soft drinks & cake**
Chairs: Kristoffer Almdal, Professor, DTU Chemistry & Günther Peters, Professor, DTU Chemistry

13:30 – 14:00 ***Proteins from the Death: Human Evolution Through the Lens of Ancient Proteins***
Frido Welker, Postdoctoral Researcher, Globe Institute, University of Copenhagen, Denmark
Chair: Ulrich auf dem Keller, Professor, DTU Bioengineering

14:00 – 14:30 ***Climbing Up and Down Binding Landscapes: a High-throughput Study of Mutational Effects in Protein-Protein Complexes***
Julia Shifman, Principal Investigator, Alexander Silberman Institute of Life Sciences, Hebrew University Jerusalem, Israel
Chair: Marie S. Møller, Assistant Professor, DTU Bioengineering

14:30 – 15:00 ***Protein Folding on the Ribosome***
John Christodoulou, Professor in Biological NMR Spectroscopy, University College London, United Kingdom
Chair: Alexander Buell, Professor, DTU Bioengineering

15:00 – 15:20 **Coffee Break**

15:20 – 15:50 ***Entropy and Enzyme Adaptation***
Johan Åqvist, Professor, Department of Molecular and Cellular Biology, Uppsala University, Sweden
Chair: Peter Westh, Professor, DTU Bioengineering

15:50 – 16:20 ***Exploring Carbohydrate Active Enzymes Diversity***
Bernard Henrissat, Research Director, Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille University, Luminy, France
Chair: Maher Abou Hachem, Professor, DTU Bioengineering

16:20 – 17:00 **End of day -“time for a beer” & posters**

List of abstracts

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2. Anne Blicher: Quantitative amino acid analysis
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4. Birgitte Zeuner: Protein engineering, enzyme discovery, and biomass valorisation for enzymatic oligosaccharide synthesis and modification
5. Christian Dybdahl Andersen: Understanding the structure-function relationship in cold-adapted enzymes
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14. Maria Dalgaard Mikkelsen: Fucoidan: Enzymatic Purification and Modification
15. Marie Sofie Møller: Carbohydrate binding module family 45 – a low affinity starch binding family
16. Matteo Lambrughi: How sphingolipids regulate the structure and dynamics of membrane proteins
17. Matteo Tiberti: MutateX: an automated pipeline for in-silico saturation mutagenesis of protein structures and structural ensembles
18. Mikkel Madsen: Mapping the Interaction site(s) of Alginate oligosaccharides on βeta-lactoglobulin
19. Mukesh Kumar: The structural and mutational landscape of autophagy proteins in cancer
20. Nanna Sandager Røjel: Systematic deletions reveal functional roles of the loops in *Trichoderma reesei* Cel7A
21. Oana N. Antonescu: Shining light on split fluorescent protein fitness landscapes using peptide array-based deep substitutional scanning
22. Sanaullah Khan: Identification and Structural Analysis of Alginate Oligosaccharide Binding Sites on β-lactoglobulin
23. Sebastian Jannick Muderspach: Further Structural Studies of the Lytic Polysaccharide Monooxygenases *Ao*AA13

24. Silke Flindt Badino: Evolutionary pressure on substrate binding in heterogeneous catalysis - Functional roles of a highly conserved asparagine residue in a loop of the processive cellulase Cel7A
25. Simonas Savickas: Hybrid degradomics: interactivity of matrix metalloproteinases and the surrounding degradome at the wound edge
26. Simone Scrima: LypidDyn: A computational microscope to scrutinize membrane properties at the organelle-level
27. Sonja Kunstmann: A flexible loop of a starch-active AA13 LPMO
28. Stefan Jarl Christensen: Rate-limiting step and substrate accessibility of cellobiohydrolase Cel6A from *Trichoderma reesei*
29. Timothy P. Jenkins: The unlocked potential of venom gland transcriptomics, droplet microfluidics, and alternative protein scaffolds
30. Valentina Perna: Laccase-catalyzed oxidation of lignin induces production of H_2O_2
31. Wahyu Wijaya: The assembly of β- and κ-casein mixed micelles: thermodynamic and structural considerations
32. Yong Wang: Integrative Ensemble Modeling of a Large Membrane Protein Complex Using Diverse and Ambiguous Information
33. Dionisio Sánchez: The mechanism of recognition and binding between a BH3-only protein and Bcl-xL using all-atom molecular simulations

Abstract no. 1

Towards improved biophysical models of protein folding to identify disease-causing mutations

Amelie Stein, Amanda B. Abildgaard, Sofie V. Nielsen, Kresten Lindorff-Larsen, Rasmus Hartmann-Petersen

Structural Biology and NMR Laboratory, Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen

The rapid decrease in DNA sequencing cost is revolutionizing medicine and science. In medicine, genome sequencing has revealed millions of missense variants that change protein sequences at a single site, yet we only understand the molecular and phenotypic consequences of a small fraction of these. Within protein science and biotechnology, high-throughput multiplexed assays enable us to probe the effects of thousands of variants in a single experiment.

We have used computational and experimental approaches to determine the consequences of missense variants in proteins, with the aim of using such models both for diagnosing genetic diseases, and for providing mechanistic insight into disease. In particular, we have focused on the effects of individual amino acid changes on protein folding and stability, linking biophysical calculations with protein degradation and abundance in cells. By examining a range of proteins and diseases we have found that loss of stability is a common driver for genetic diseases, and that predictions of changes in thermodynamic protein stability are useful to assess the pathogenicity of genetic variation. I will discuss these ideas using recent examples from our laboratories.

At the same time, our work has also revealed areas where our understanding and ability to predict the effect of amino acid changes is still imperfect. I will discuss how we are using sequence analyses and high-throughput experiments to understand the origin of these effects, thus paving the way for more accurate biophysical models for use in personalized medicine.

References

Abildgaard, Amanda B., et al. "Structural destabilization and chaperone-assisted proteasomal degradation of MLH1 as a mechanism for Lynch syndrome." bioRxiv (2019): 622266.

Clausen, Lene, et al. "Protein stability and degradation in health and disease." Advances in protein chemistry and structural biology 114.1 (2019): 61-84.

Kampmeyer, Caroline, et al. "Blocking protein quality control to counter hereditary cancers." Genes, Chromosomes and Cancer 56.12 (2017): 823-831.

Nielsen, Sofie V., et al. "Predicting the impact of Lynch syndrome-causing missense mutations from structural calculations." PLoS genetics 13.4 (2017): e1006739.

Scheller, Rasmus, et al. "Toward mechanistic models for genotype–phenotype correlations in phenylketonuria using protein stability calculations." Human mutation 40.4 (2019): 444-457.

Stein, Amelie, et al. "Biophysical and Mechanistic Models for Disease-Causing Protein Variants." Trends in biochemical sciences (2019).

Abstract no. 2

Quantitative amino acid analysis

Anne Blicher & Katja S. Johansen

Biomass Science and Technology Research Group, Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg, Denmark

E-mails: abl@ign.ku.dk; ksj@ign.ku.dk

Amino acid analysis determines the amount of protein in a sample and the composition, i.e. ratios between the different amino acids. Quantitative amino acid analysis is the only method for determination of the absolute amount of a protein. The composition, presented as mol% can be used for identification of a protein from databases. The analyses consist of chromatographic separation of all amino acids in the protein and quantification of each of them. For proteins and peptides this separation requires hydrolysis of peptide bonds to obtain the free amino acids.

A method based on normal phase separation with post column derivatisation of the amino acids is now implemented. This exact same method was previously running at Protein and Immune Systems Biology, DTU. Data for samples analysed on this newly purchased systems are in complete agreement with data from the old system.

We can now offer to carry out quantitative amino acid analysis on your samples. Detailed information about requirements to the samples and effect on the different amino acids during hydrolysis will be given at the poster.

Abstract no. 3

The influence of N-glycosylation on the activity of cellobiohydrolase Cel7A from *Trichoderma reesei*: application of the inverse Michaelis-Menten equation

Bartłomiej Kołaczkowski[1], Kenneth Jensen[2], Kristian B. R. M. Krogh[2], Peter Westh[3]

[1]Roskilde University, Department of Science and Environment,1 Universitetsvej, DK-4000, Roskilde, Denmark
[2]Novozymes A/S, Krogshøjvej 36, DK-2880, Bagsværd, Denmark
[3]Technical University of Denmark, Department of Biotechnology and Biomedicine, Søltofts Plads, 2800 Kgs. Lyngby Denmark

Glycosylation is a commonly observed post-translational modification of fungal cellulases. The typical architecture of cellulases includes N-glycosylated catalytic domain, which is connected to carbohydrate-binding module through a flexible, O-glycosylated linker peptide.

Functional roles of the N-glycosylation have been widely studied but remain elusive. Most attempts to elucidate effects of glycosylation on cellulase activity have used end-point measurements or conventional Michaelis-Menten theory, which may not be fully descriptive if the cellulase activity is tested on insoluble substrate.

In the current work, we have studied effects of glycans on cellulase activity using a broader kinetic approach. Specifically, we investigated the wild type and an N-glycan knockout of the cellobiohydrolase Cel7A from *T. reesei*. Our kinetic analysis included both substrate saturation (E0 << KM + S0; conventional MM) and enzyme saturation (S0 << KM + E0, inverse MM), and the results revealed an interesting higher number of attack sites on the cellulose surface and changed binding capacity for the N-glycan mutant.

There is a further need of characterizing single N-glycan mutants of the Cel7A. Also, it is important to investigate glycan structures and try to understand the correlation between the location/composition of each N-glycan present on Cel7A and its activity on insoluble cellulose.

Abstract no. 4

Protein engineering, enzyme discovery, and biomass valorisation for enzymatic oligosaccharide synthesis and modification

Birgitte Zeuner*, Marlene Vuillemin, Jan Muschiol, Jesper Holck, Anne S. Meyer

Protein Chemistry and Enzyme Technology, Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800 Kgs. Lyngby.

*bzeu@dtu.dk

Brief description of research area:
Through reaction optimization and protein engineering, glycoside hydrolases (GHs) can be brought to efficiently catalyse transglycosylation for oligosaccharide synthesis and modification. The advantages of using GHs include easy expression and the ability to use inexpensive, naturally abundant substrates, e.g. agro-industrial side streams. During the past decade, our focus has been on enzymatic synthesis of human milk oligosaccharide (HMO) structures. HMOs are unique oligosaccharides, which are essential for infant health and development, but essentially absent from the bovine milk used for infant formula production. An example of biomass valorisation for this purpose includes the use of casein glycomacropeptide (CGMP) as sialyl donor. Our poster presents characterization and engineering of GH29 α-L-fucosidases for synthesis of fucosylated HMO structures, including utilisation of fucosylated xyloglucan extracted from citrus peel. From here, we would like to expand our technology to cover other oligosaccharides or glycans.

What we know:

- Enzyme-catalysed transglycosylation for oligosaccharide synthesis
- Analysis of reactions with glycosidase-catalysed oligosaccharide synthesis (HPAEC-PAD, LC-MS)
- Human milk oligosaccharide (HMO) synthesis and analysis
- Protein engineering for improved transglycosylation/reduced hydrolysis
- Enzyme discovery for transglycosylation

What we need:

- Chemical synthesis and/or NMR for structural elucidation of regioisomers

Abstract no. 5

Understanding the structure-function relationship in cold-adapted enzymes

Christian Dybdahl Andersen[1], Jan S. Nowak[1], Mariane S. Thøgersen[2], Peter Stougaard[2] and Daniel Otzen[1].

[1]Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark.
[2]Department of Environmental Science, Aarhus University, Roskilde, Denmark.

Background:
Enzymes are widely used in industrial processes, commercial products and in biotechnology. However, most of these enzymes are meso- or thermophilic with optima around 35-55°C and 55-70°C respectively. Several applications for enzymes with lower temperature optima exist. Examples include (a) the food industry where processing takes place at low temperatures due to food safety and quality and (b) biotechnology where inactivation of enzymes at lower temperatures would ease downstream processes. Psychrophilic enzymes have been identified in bacteria isolated from cold environments. Unlike meso- and thermophilic enzymes, psychrophilic enzymes show high activity at low temperatures (0-20°C). Furthermore, psychrophilic enzymes are heat-labile and can be inactivated at low temperatures.

What we know:
In order to compensate for the decrease in catalytic activity at low temperatures, cold-active enzymes adapt to a more flexible structure resulting in a decrease of the free energy of activation. Several structural adaptions, as compared to meso- and thermophilic homologs, have been observed in psychrophilic enzymes. These include loop extensions, changes in the number of disulfide bridges, reduction in the number of hydrogen bonds and an increase in surface exposed hydrophobic residues. To date, however, no clear strategy for cold-adaption has been elucidated.

What we need:
With a library of novel bacteria isolated from permafrost environments as a starting point, we mine for metabolically important and industrial relevant enzymes showing high activity at low temperatures. These include (a) triosephosphate isomerases and lactate dehydrogenases, which are essential in the metabolism (b) alpha-amylases, beta-galactosidases and catalases for the food industry and (c) endonucleases and alkaline phosphatases for biotechnology. The aim of this project is to study the structure-function relationship of selected enzymes in order to understand the structural adaptions leading to cold-activity. Understanding this relationship will allow for the identification of novel industrially relevant psychrophilic enzymes and engineering of cold-adaption into already commercialized enzymes. This, however, requires deeper insight into the strategies of cold-adaption e.g. between different fold classes. This can be accomplished by a combined study of psychrophilic enzyme activity, structure, flexibility and dynamics as compared to meso- and thermophilic homologs.

Abstract no. 6

Systematic deletions in *Trichoderma reesei* Cel7A reveal functional roles of the flexible loops

Corinna Schiano di Cola[1], Nanna Røjel[1], Kenneth Jensen[2], Jeppe Kari[1], Kim Borch[2] and Peter Westh[1,3]

[1]Research Unit for Functional Biomaterials, Department of Science and Environment, Roskilde University, Universitetsvej 1, DK-4000, Roskilde, Denmark
[2]Novozymes A/S, Rævehøjvej 32A, DK-2800, Lyngby, Denmark
[3]Section for Protein Chemistry & Enzyme Technology, DTU Bioengineering, Technical University of Denmark, Søltofts Plads, DK-2800, Lyngby, Denmark

Brief description of research area:
Fungal cellulases from glycoside hydrolase family 7 (GH7) are some of the most efficient enzymes for cellulose degradation. They are highly relevant for industries producing sustainable fuels and chemicals from lignocellulosic biomass feedstocks. The cellulolytic model fungus *Trichoderma reesei* secretes two major synergistic GH7 enzymes: the first is an exo-acting, processive cellobiohydrolase, TrCel7A, and the second is an endoglucanase with preference for amorphous cellulose, TrCel7B. Structurally, they both contain a linker region and a carbohydrate-binding module, and they mostly differ in the catalytic domain, where TrCel7A displays a tunnel-shaped active site formed by eight peripheral loops (A1-A4, B1-B4), while TrCel7B has a more open cleft shape due to the absence of loops B2, B3, B4 and A4.

What we know:
To investigate the structure-function relationships of the loops missing in TrCel7B, we produced and kinetically characterized a family of variants in which four unique loops in TrCel7A were individually deleted in order to resemble the structure of TrCel7B1. Analysis of a range of kinetic parameters consistently showed that the B2 loop, covering the pyranose-binding subsites -3 and -4 in TrCel7A, was a key determinant for the distinction between a more cellobiohydrolase- or endoglucanase-like behavior.

What we need:

- We appreciate collaborations with computational chemists and structural biologists to further analyse the effects of structural modifications on cellulases.
- We would like to apply our enzyme kinetic approaches on other interfacial enzymes.

References:
1 Schiano-di-Cola, C., Røjel, N., Jensen, K., Kari, J., Sørensen, T.H., Borch, K. and Westh, P., 2019. Systematic deletions in the cellobiohydrolase (CBH) Cel7A from the fungus Trichoderma reesei reveal flexible loops critical for CBH activity. Journal of Biological Chemistry, 294(6), pp.1807-1815.

Abstract no. 7

Xyloglucan utilization locus in *Bifidobacterium dentium* bd1

Deniss Petrovs[1], Jens Vogensen[1,2], Erwin Schoof[3], Birte Svensson[1]

[1] Enzyme and Protein Chemistry, Department of Bioengineering, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.
[2] Cell Biology and Physiology, Department of Biology, University of Copenhagen, DK-2200 Copenhagen, Denmark.
[3] DTU Proteomics Core, Department of Bioengineering, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

Brief description of research area:
The human gastrointestinal tract is colonized by a dense microbial ecosystem, which is responsible for the digestion of complex polysaccharide dietary fibers consumed by humans from a wide range of foods. Xyloglucan is a prominent hemicellulosic polysaccharide constituting up to 25% of common vegetable dry weight. We have identified a xyloglucan utilization locus (XgUL) in *Bifidobacterium dentium* bd1 that belongs to the *Bifidobacterium* genus, known for its probiotic properties. The *B.dentium* bd1 XgUL is the yet most complex encoding 8 glycoside hydrolases (GHs) belonging to 6 GH families, 6 ABC transporter proteins and an esterase. Comparative proteomic analysis with label-free quantification identified >40 differentially abundant proteins from 1254 high-confidence protein identifications. The detailed molecular investigation of the XgUL of *B.dentium* bd1 will expand our understanding of the sophisticated carbohydrate degradation systems that gut microbes have developed in the competitive gut environment.

What we know: molecular cloning, protein production, expression, purification, characterization

What we need: bacterial growth competition analysis, polysaccharide isolation and purification

Abstract no. 8

Elucidating the structure-function relationship of cryophilic enzymes

Jan S. Nowak[1], Christian D. Andersen[1], Marianne S. Thøgersen[2], Peter Stougaard[2], Daniel Otzen[1]

[1]Interdisciplinary Nanoscience center (iNANO), iNANO-MBG, Aarhus University, Gustav Wields Vej 14, 8000 Aarhus C, Denmark
[2]Department of environmental sciences, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark

Abstract:
Industrial processes utilizing enzymatic reactions are often required to be performed under chilly conditions due to e.g. food safety, product quality or simply the economics of production. Such enzymes include e.g. β-galactosidases used in making diary-free milk production and lipases in biofuel development. As most commercial enzymes are derived from meso- and thermophilic organisms, they tend to have temperature optima around 35-55°C and 55-70°C, respectively. Psychrophilic enzymes are on the other hand are highly active around 0-20°C due to evolutionary adaptations of microorganisms inhabiting arctic environments. However, because of the inherent flexibility that grant them the low-temperature properties, these enzymes suffer in terms of stability making them less than ideal for commercial use. In this study, we seek to discover and characterize housekeeping enzymes from unique cryophilic microorganisms to pinpoint cold-activity determinants and produce robust, industry-friendly enzymes.

What we know:
Microorganisms that are capable of growth at cold environments such as the permafrost require adaptation on all levels, including the most basic molecular mechanisms of a cell. Comparing these to their mesophilic and thermophilic counterparts, the cold-active adaptations often include a more fluid cell membrane, chaperone expression, increased nutritional uptake, optimized resource distribution and enzyme sequence alterations.

The low temperature of permafrost environments provides the cryophilic bacteria with a thermodynamic challenge of sustaining metabolic activity in spite of it the low temperatures. Several subtle changes have been reported on how these cryophilic bacteria implement cold-adaptation to their enzymes, which among other include decreased number of salt bridges on the surface of protein, extended loops, and a reduction in core hydrophobicity, fewer disulfide bridges and decrease in total number of hydrogen bonds. Compared to meso- and thermophiles the overall picture paints a more flexible structure particularly affecting the dynamics of the catalytic site, even though it is typically conserved in terms of amino acid composition.

This reveals the main strategy of cold-adaptation to achieve high turnover at low temperatures, as the increased flexibility leads to weaker substrate binding which lowers the activation barrier through a shift from an enthalpic cost to an entropic one, which in turn is less affected by temperature. However, the flexibility of cryophilic enzymes also comes with consequences to their stability, as these tend to denature at moderate temperatures. Interestingly, where the

meso- and thermophilic homologs tend to lose their activity as the three-dimensional structure unfolds, the activity of cryophilic enzymes seems be affected before the unfolding events are measured.

What we need to know:
No clear strategy for cold-adaptation have been identified yet. Our goal is to identify and characterize primary metabolic cold-adapted enzymes from bacteria isolated in unique permafrost environments. We wish to gain insight into structural determinants for cold-activity based on comparative studies of cryophilic, mesophilic and thermophilic housekeeping enzymes. Understanding the structure-function relationship of cryophilic enzymes will aid us in identification and bioengineering of industrially relevant enzymes based on the gained knowledge from the housekeeping enzymes.

Abstract no. 9

Web-visualization of dynamic structural data for interdisciplinary research

Johanna K. S. Tiemann[1], Alexander S. Rose[2], Peter W. Hildebrand[1,3]

[1]*Institute of Medical Physics and Biophysics, University Leipzig, Germany*
[2]*RCSB Protein Data Bank, San Diego Supercomputer Center, University of California, USA*
[3]*Institute of Medical Physics and Biophysics, Charité – Medical University of Berlin, Germany*

Brief description of the research area:
Molecular dynamics (MD) simulations are these days a well-established and widely used technique to investigate time-resolved motions of macromolecules such as membrane proteins like G protein-coupled receptors (GPCR), one of the pharmacologically most relevant receptor class. Sharing of the resulting coordinate trajectories even for visualization is complicated by their size, the requirement of having special software set-up and the knowledge of the software usage. However, in light of increasing interest in the investigation of time-resolved motions in interdisciplinary research and remote collaborations, it is desirable to make sharing of MD trajectories easier to facilitate discussions and further analyses.

By providing access via the web, tools to serve and visualize trajectories of molecular coordinates like the MDsrv [1] (for example cases see http://nglviewer.org/mdsrv) bridge the gap between computational and experimental researchers and even promote concepts for publishing MD data along with publications of their analysis. The concept of visualizing and sharing of MD trajectories via the web can be considered as a major step of the MD field towards a community following the FAIR (Findable, Accessible, Interoperable, Re-usable) principles. This process will be significantly guided by the usage of visual sharing tools like MDsrv [2].

What I know:
Methods & techniques:

- Secondary structure & interaction prediction
- Modeling using bioinformatics methods with the inclusion of cryo-electron microscopy densities
- Classical molecular dynamics (MD) simulations & enhanced sampling techniques
- Development of modeling, visualization, sharing, and analysis tools

Main biological systems (currently):

- Membrane proteins, esp. G protein-coupled receptors
- Membrane associating proteins such as G proteins, Arrestins, Kinases
- Combination of them

What I am interested in:

- Influences of genomic variations on the structure and dynamic function of proteins
- Investigation of intermediate/transient states & their context in the dynamic equilibrium
- Crowded cells, including complex membranes and their dynamics

- Accessibility, sharing, and re-usage of esp. data from MD
- Algorithmic developments for the modeling & analysis of structural data, e.g. from MD

What I need:

- For experimental collaborations & data interpretation: a better understanding of their techniques (benefits & limitations) & their needs for interdisciplinary research
- Connecting sequence data like variations & mutations with functions & dynamics of proteins

References:

[1] Tiemann, J. K. S., Guixà-González, R., Hildebrand, P. W., & Rose, A. S. (2017). MDsrv: Viewing and sharing molecular dynamics simulations on the web. Nature Methods, 14(12), 1123–1124. https://doi.org/10.1038/nmeth.4497

[2] Hildebrand, P. W., Rose, A. S., & Tiemann, J. K. S. (2019). Bringing Molecular Dynamics Simulation Data into View. Trends in Biochemical Sciences, in Press. https://doi.org/10.1016/j.tibs.2019.06.004

Abstract no. 10

Free Energy Calculations of Cellulases

Kay Sven Schaller[1], Gustavo Avelar Molina[1], Jeppe Kari[1], Günther H.J. Peters[2], Kim Borch[3], Peter Westh[1]

[1]Department of Biotechnology and Biomedicine, Technical University of Denmark
[2]Department of Chemistry, Technical University of Denmark
[3]Novozymes A/S, Denmark

Brief description of research area:
Our group concerns itself with interfacial enzyme catalysis with focus on cellulases. The goal of this project is to apply the Sabatier's principle for enzyme catalysis, increase understanding of heterogeneous biocatalysis and exploit arising possibilities for enzyme design.

What we know:
Cellulases, Molecular Dynamics, free-energy calculations…

What we need:
Cheap computational methods for binding strength prediction, other use cases…

Abstract no. 11

Snake venom proteinase activity profiling through screening of peptide substrates

K. Kalogeropoulos[1], A.F. Treschow1, U.a.d. Keller[1], T. Escalante[2], A. Rucavado[2], J.M. Gutiérrez[2], A.H. Laustsen[1] and C.T. Workman[1]

[1]Department of Biotechnology and Biomedicine, Technical University of Denmark, 2800 Lyngby, Denmark
[2]Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José 11501-2060, Costa Rica

Metalloproteinases and serine proteinases are among the most abundant enzymes in many snake venoms, particularly among viperids. These proteinases are responsible for some of the clinical manifestations classically seen in viperid snakebites, including hemorrhage, necrosis, and coagulopathies.

The study investigated the activities of said proteinases using a rich peptide library of biologically relevant proteins, as a method of screening for the target substrates of the venom proteinases of five viperid (*Echis carinatus*, *Bothrops asper, Daboia russelii, Bitis arietans, Bitis gabonica*) and one elapid (*Naja nigricollis*) species of high medical importance. The venoms were each tested against 360 peptide substrates, yielding 2160 enzymatic activity profiles. A nonlinear regression model that accurately described the observed activities was constructed based on the experimental data, allowing for cleavage rate comparison across species.

In this study, previously unknown protein targets of snake venom proteinases were identified, potentially implicating novel human and animal proteins that may be involved in the pathophysiology of snake envenomings. These new findings may contribute to our understanding of the clinical manifestations and underlying biochemical mechanisms of snakebite envenoming by the investigated snake species.

Abstract no. 12

Peptide-Signature Grouping of CAZymes – for Biomass Conversion

Kristian Barrett[1], Jesper Holck[1], Anne S. Meyer[1] & Lene Lange[2]

[1]Department for Biotechnology and Biomedicine, Building 221, Technical University of Denmark, DK-2800 Lyngby, Denmark
[2]LLa Bioeconomy, Research & Advisory, Karensgade 5, DK-2500 Valby, Denmark

Brief description of research area:
Insight into the function of CAZymes is an important prerequisite for understanding their biological significance and forms a decision base for selection of enzymes for potential industrial use. However, it would involve an insurmountable amount of work to experimentally characterize all known CAZymes. Therefore, there is a need to define groups of enzyme proteins likely be functionally similar. For such grouping to be viable it needs to be robust over time while being dynamic enough to embrace the most recent proteins as they come.

Peptide-signature grouping is a new approach for sequence analysis of enzymes. This method relies upon non-alignment based similarities of short peptide sequences, i.e. identification of Conserved Unique Peptide Patterns (CUPP). CUPP has been developed to perform automated clustering of proteins for formation of protein groups Barrett & Lange, 2019. The analysis is based on the hypothesis that conserved peptide patterns (peptide-signatures) can be used to identify similarities between proteins and create groups. Such defined distinct protein groups can be used to chart the divergence within CAZy families. The CUPP groups created by unique peptide-signatures have been shown to group CAZyme-proteins having similar functions.

We have shown that the unique peptide signatures identified within each of the CUPP groups can furthermore be used for fast annotation of enzyme proteins to assign family, subfamily and molecular function as EC number of carbohydrate-active enzymes.

What we know:
Natural carbohydrates in plants are much more complex than the details captured in the CAZy family and subfamily delineation or included in a molecular function described as an EC number. A much more functionally nuanced organization of the CAZymes below CAZy family level can enhance the ability to interpret such relationships between CAZymes.

What we need:
Acquisition of transcriptomics and proteomics experimental data from growth experiments of microbes on natural substrates to better understand the interplay between groups of CAZymes acting on their natural substrates. Furthermore, detailed characterization data and insight from experiments where CAZymes have been tested on different kinds of natural substrates including their respective natural substrates through a systematical approach.

Reference: Barrett K, Lange L. Peptide-Based Classification and Functional Annotation of Carbohydrate-Active Enzymes by Conserved Unique Peptide Patterns (CUPP). Biotechnol Biofuels. BioMed Central; 2019; 1–21.

Abstract no. 13

Solution structures of albumin-detemir complexes studied by small-angle X-ray scattering and *in-silico* modelling

L. A. Ryberg[1], P. Sønderby[1], F. Barrientos[1], J. T. Bukrinski[2], P. Harris[1] and G. H. J. Peters[1].

[1]*Technical University of Denmark, Department of Chemistry, 2800 Kgs. Lyngby, Denmark.*
[2]*CMC Assist Aps, 2500 Copenhagen, Denmark.*

Brief description of research area:
Protein drugs are increasingly important in drug development worldwide due their high specificity, potency, and low toxicity. Many protein drugs do, however, suffer from inherent physical instability limiting the manufacturability and formulation development, as well as short plasma half-lives that are incompatible with delivery of an efficacious dose within the appropriate dose regime[1,2].

Human serum albumin (albumin) can be an approach to solving both challenges. Firstly, recombinant albumin can be used as an excipient to stabilize drug formulations (1). Secondly, protein drugs may be designed to bind to albumin in vivo and thereby extend their half-lives. An example is the acetylated insulin analogue, insulin detemir (detemir) (2).

What we know:
In this work, complex formation between albumin and detemir and how it is influenced by physicochemical properties in different formulations have been investigated using an interdisciplinary approach that combines light scattering, small-angle X-ray scattering (SAXS), size exclusion chromatography (SEC) and molecular dynamics (MD) simulations. SAXS has been measured both in standard batch and in SEC-SAXS mode to obtain monodisperse data from polydisperse samples. Based on the data, *ab-initio* and rigid body modelling have been carried out to obtain solution structures of albumin-detemir complexes. As rigid body modelling does not take chemical information into account, the complexes were further investigated by MD simulations.

Acknowledgements:
MAX IV and EMBL Hamburg are acknowledged for providing beam time for the SAXS experiments. Albumedix Ltd. is acknowledged for providing protein for the experiments.

References:
1 M. Perkins, *BIOPHARM INT* **25**, 3 (2012).
2 D. Sleep, *Expert Opin. Drug Deliv.* **12**, 5 (2016).

Abstract no. 14

Fucoidan: Enzymatic Purification and Modification

Maria Dalgaard Mikkelsen[1], Hang T. T. Cao[1,2], Thuan N. Thi[1,2], Trang V. T. Dieu[1,2] , Jan Muschiol[1], Anne S. Meyer[1]

[1]Enzyme Technology, Section for Protein Chemistry and Enzyme Technology, Department of Biotechnology and Biomedicine, Technical University of Denmark
[2]NhaTrang Institute of Technology Research and Application, VAST, Vietnam

The cell walls of brown algae contain fucoidan, a polysaccharide made of mainly sulfated fucose residues. The structural diversity of fucoidan is extremely large due to varieties in monosaccharide compositions, different types of glycosidic linkages and the presence of large numbers of non-carbohydrate substituents, like sulfations and acetylations.

Fucoidans have been extensively studied because of their wide range of biological activities, such as anticoagulant, antithrombotic, anticancer, anti-inflammatory and immunomodulatory [1-4]. No drug is although currently available due to the heterogeneity of the fucoidans.

Fucoidan-modifying enzymes, including fucoidanases and sulfatases, might be the best tool for not only determining the fine-structures of fucoidans but also to determine the relationship between fucoidan and the biological activities; hence enzymes might be the best way to enable the production of homogenous fucoidan drugs. Furthermore enzymes can also be used, instead of chemicals, to extract fucoidans from seaweeds in a much more green and gentle way.

References:

[1] Ale, M.T. et al. 2011. Int. J. Biol. Macromol. 49, 331–336.
[2] Ermakova, S.P. et al. 2015. J. Appl. Phycol. 28, 2495–2505.
[3] Lapikova, E.S. et al. 2008. Bull. Exp. Biol. Med. 146, 328–333.

Abstract no. 15

Carbohydrate binding module family 45 – a low affinity starch binding family

Marie Sofie Møller, Birte Svensson

Enzyme and Protein Chemsitry, Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, Denmark.

E-mail: msm@bio.dtu.dk

Brief description of research area:
Interactions between proteins and carbohydrates play a vital role in life. Through evolution, these interactions have been optimized to match the environment wherein they take place. The binding affinities of protein–carbohydrate complexes range from mM to sub-µM. The low affinity interactions (mM range) are hypothesised to play a role in dynamic systems e.g. in relation to plant cell wall synthesis and degradation, and microbe-host interplay. Here work on carbohydrate binding module family 45 members is presented. Carbohydrate binding module family 45 (CBM45) has been shown to habour starch binding domains of low affinity. CBM45s are occurring as tandem repeats in a small number of plant proteins, i.e. plastidial α-amylase and starch phosphorylating glucan, water dikinases (GWDs).

What we know:
We analyse protein-carbohydrate binding using different techniques including; surface plasmon resonance (SPR), pull-down assays with insoluble substrates, and affinity gel electrophoresis.

What we need:
Additional techniques suitable for analysing low affinity interactions between soluble polysaccharides and the CBMs, since the conditions of affinity gel electrophoresis can make some CBMs unstable.

Acknowledgements
The work was supported by the Independent Research Fund Denmark (FNU).

Abstract no. 16

How sphingolipids regulate the structure and dynamics of membrane proteins

Matteo Lambrughi[1], Elisabeth Corcelle-Termau[2], Simone Scrima[1], Kenji Maeda[2], Marja Jäättelä[2], Elena Papaleo[1,3]

[1]Computational Biology Laboratory, Center for Autophagy, Recycling and Disease, Danish Cancer Society Research Center, Copenhagen, Denmark
[2]Cell Death and Metabolism Unit, Center for Autophagy, Recycling and Disease, Danish Cancer Society Research Center, Copenhagen, Denmark
[3]Translational Disease Systems Biology, Faculty of Health and Medical Sciences, Novo Nordisk Foundation Center for Protein Research University of Copenhagen, Copenhagen, Denmark.

Abstract:
Sphingomyelins (SM) are structural components of membranes and play a crucial role in defining organelle identity and cellular functions. Lipids modulate the structure, dynamics, and confinement of membrane proteins, exerting a major regulatory mechanism on their biological activities. A proper organization of SM is required for trafficking of membrane proteins between the different cellular organelles, such as the autophagic protein ATG9A [1-2], and they can act to modulate their biological activities, as p24 [3]. Nevertheless, the molecular details of the binding networks and recognition of specific species of lipids with the membrane proteins are still elusive. The comprehension of lipid-protein relationships is of primary importance since their alterations are associated with cancer, representing highly prospecting target mechanisms for design treatments.

What we know:
Our group (Computational Biology Laboratory, at the Danish Cancer Society Research Center, DCRC) is focused to elucidate the molecular mechanisms behind multiple aspects of protein-lipid interactions, how they are deregulated in cancer and effects of small-molecules with therapeutic potential. We are using experimentally-driven computational methods, bringing together data from cell biology, biochemistry and lipidomics of single organelles, thanks to the collaboration with the group of Prof. Jäättela at the DCRC. With this approach, we are investigating the structure, dynamics and lipid-based modulation of ATG9A and other membrane proteins in different organelle-like environments. We are exploring the binding of SM species with membrane proteins, shedding light on specific lipid-protein interactions that can be related to functional mechanisms and how they are perturbed in cancer.

What we need:
Molecular simulations are promising to describe the structural biology of lipid membranes and proteins [4]. While experiments generally permit to observe spatial and temporal average properties, molecular simulations can provide atom level and detailed properties that are essential to rationalize them. On the other hand, computational data need to be accurately integrated and validated by experiments. In our group we are seeking novel experimental collaborators, experts in biophysical techniques to investigate lipid membranes and interactions

with proteins as NMR spectroscopy, electron microscopy, mass spectrometry, and optical spectroscopies.

Acknowledgments:
This research is supported by the Danish Council for Independent Research, Natural Science Research Project 1 (102517) and by the Center of Excellence in Autophagy Recycling and Disease (CARD), funded by the Danish National Research Foundation. The calculations were performed using resources provided by the DeiC National Life Science Supercomputer at DTU.

References:
[1] Corcelle-Termeau et al. Autophagy 12 833–849 (2016)
[2] Björkholm et al. B.B.A. 1838 2066–2070 (2014)
[3] Contreras et al. Nature 481 525–529 (2012)
[4] Vattulainen and Róg, B.BA. 1858, 10, 2251-2253 (2016)

Abstract no. 17

MutateX: an automated pipeline for in-silico saturation mutagenesis of protein structures and structural ensembles

Matteo Tiberti[1], Thilde Terkelsen[1], Tycho Canter Cremers[1], Miriam Di Marco[1], Isabelle da Piedade[1], Emiliano Maiani[1], Elena Papaleo[1,2]

[1]Computational Biology Laboratory, Center for Autophagy, Recycling and Disease, Danish Cancer Society Research Center, Copenhagen, Denmark
[2]Translational Disease Systems Biology, Faculty of Health and Medical Sciences, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark.

Brief description of the research area:
Genomic missense mutations resulting in amino acid substitution affect critical aspects of how proteins work, such as structural stability, dynamics, function and binding to other biomolecules. Mutant variants of key genes can be fundamental drivers for the development of cancer, even though most of the somatic mutations do not contribute to malignant initiation or progression [1]. Our group at the Danish Cancer Society Research Center (DCRC) has been focusing on approaches based on the structure, function and dynamics of the protein products to classify the effects of mutations found in cancer samples, using computational methods.

What we know:
One of the main areas of interest for our group is understanding the effects of mutations on protein stability and protein-protein interactions. In-silico saturation mutagenesis can be a suitable tool to guide the design of new experiments or rationalize at the atomic level the consequences of mutations. Here we introduce MutateX, a software pipeline that aims at automating the prediction of differences in free energy associated with the systematic mutation of each available residue within a protein or protein complex, by employing the FoldX [2] energy function in a high-throughput scenario. Our deep mutational scans allow to identify mutational hot spots affecting the structural stability or protein-protein interactions and rationalize experimental results in the context of deep mutational scans. Such calculations can be performed on a single or on an ensemble of protein conformations, either from experiments or simulations, to take into account different conformational substrates.

What we need:
We are thus looking for i) collaborators interested in trying out our software as end-users or interested in trying our approach on their system(s), ii) researchers willing to validate our findings by means of experiments using for example in vitro biochemical assays, iii) researchers developing other methods for the estimation of the effect of mutation on protein stability or protein-protein interaction, that we could integrate with our software in a broader scenario.

Acknowledgments:
The project was supported by Danmarks Grundforskningsfond (DNRF125), a Carlsberg Foundation Distinguished Fellowship (CF18-0314), a LEO foundation exploratory grant (LF17006), and Innovationsfonden (5189-00052B). The calculations described in this paper

were performed using the DeiC National Life Science Supercomputer Computerome at DTU (Denmark), and DECI-PRACE 14th HPC Grant for calculations on Archer (UK).

References:

[1] J. R. Pon and M. A. Marra. "Driver and passenger mutations in cancer." Annual Review of Pathology: Mechanisms of Disease 10 (2015): 25-50.
[2] J. Delgado, L. G. Radusky, D. Cianferoni and L. Serrano "FoldX 5.0: working with RNA, small molecules and a new graphical interface." Bioinformatics (2019).

Abstract no. 18

Mapping the Interaction site(s) of Alginate oligosaccharides on βeta-lactoglobulin

M. Madsen[1], B. B. Kragelund[2], F. L. Aachmann[3] & B. Svensson[1]

[1]Enzyme and Protein Chemistry - Department of Biotechnology and Biomedicine – Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.
[2]SBiNLab - Department of Biology - University of Copenhagen, DK-2200 Copenhagen N, Denmark.
[3]Department of Biotechnology and Food Science – NOBIPOL, Norges Teknisk-Naturvidenskabelige Universitet, 7491 Trondheim, Norge.

Background:
Beta-lactoglobulin (βLG) is an 18.3 kDa protein found in (among others) cow milk, that has been extensively studied for its ability to bind hydrophobic ligands. It folds into nine β-strands along with an α-helix. The structure contains two sulfur bridges but also has one free cysteine buried in the hydrophobic core. The structure of βLG is highly dependent on the pH of the environment. At low pH <3 βLG is mostly a monomer, at pH >3 it is shown to be a dimer and around pH 7.5 there is movement between the open and closed state of the calyx that can bind different hydrophobic ligands. Alginate oligosaccharides are short oligomers of alginate, alginate consist of a mixture of linked β-D-mannuronic and α-L-guluronic acid either in blocks or mixed. These sugars are 1-4 linked and there is no biological system governing the composition of alginate. Alginate has a pKa around 3.4-3.6 but maintains a negative zeta potential even at pH<pKa, this is one of the reasons why it is a good binding partner for proteins. Both alginate and βLG is widely used as a food additive for gelling effects, pH modulation, water retention and antimicrobial activity, lately people have started becoming interested in the exact binding motif of the two in order to understand carbohydrate and protein interactions. This study will work with tailored alginate oligosaccharides to investigate the difference between mannuronic acid, guluronic acid, the alternating structure as well as different lengths of polymers, from dp 4 to dp 6. After we move towards understanding the binding with full length alginate and determining how the molecules pack on a macromolecular scale.

What we know:
Protein carbohydrate interactions on the molecular level. Protein characterization and structure determination. Protein NMR and carbohydrate NMR.

What we need:
Spectroscopic techniques to determine the differences in complex formation when working with larger complexes (nm-μm).

Abstract no. 19

The structural and mutational landscape of autophagy proteins in cancer

Mukesh Kumar[1], Valentina Sora[1], Burcu Aykac Fas[1], Matteo Lambrughi[1], Matteo Tiberti[1], Emiliano Maiani[1], Vladimir Rogov[2], Francesco Cecconi[3], Elena Papaleo[1,4]

[1]Computational Biology Laboratory, Danish Cancer Society Research Center, Copenhagen, Denmark
[2]Institute of Biophysical and Center for Biomolecular Magnetic Resonance, Goethe University, Germany
[3]Unit of Cell Stress and Survival, Danish Cancer Society Research Center, Copenhagen, Denmark
[4]NNF Center for Protein Research, Copenhagen, Denmark

Brief description of research area:
Autophagy is a highly conserved and coordinated intracellular clearance and recycling mechanism of cellular debris, damaged proteins, and toxins to maintain the homeostasis of cells. Autophagy has been shown to affect tumor cell survival, heterogeneity, genome instability, tumor relapse, and chemotherapeutic resistance (1). Experimental studies and bioinformatics analyses reported several alterations in autophagy genes, in different types of tumors. Such alterations in autophagy genes and the corresponding protein products could lead to structural changes, which may be linked to their dual role (i.e., as tumor suppressors or tumor promoters) in cancer.

Our goal is to investigate the specific genomic, transcriptomics and proteomic signature of a literature-based curation of key autophagy genes and proteins using bioinformatics in different tumor, subtypes, and stages. We will further explore the most interesting candidates at the structural level to unveil the related mechanisms and how cancer alterations affect them. Our data could help to identify possible hot spots for therapeutic intervention or diagnostic/prognostic markers.

What we know:
In this context, we started to curate a list of key autophagy genes from literature and divide them into different functional classes, such as ‘core-autophagy’ genes, and autophagy transcriptional regulators. We have been applying a Pan-Cancer approach to The Cancer Genome Atlas datasets, which are a rich source of cancer genomic and transcriptomic data, focusing, for now, on the core autophagy. In these analyses, we observed that core autophagy genes are often down-regulated in most cancer types with the exception of ATG9B. We are applying the same protocol to other functional autophagy classes, such as autophagy genes associated with oxidative stress transcription regulators. More than 30 recurrent missense mutations in the coding region have been found in cancer samples for each of the LC3 genes (2) and ULK1 (3).

We developed a framework accounting for different aspects of protein function and stability to understand and classify the effects of these mutations on the protein product. In particular, we included properties such as the 1) co-occurrence of mutations in the LIR- containing inter-actors

for LC3B and ULK1 proteins, ii) free energy changes associated with protein stability, and iii) changes in binding free energies for the LC3-LIR complexes. We then made a ranking based on these scores to predict possibly pathogenic mutations in LC3B and ULK1.

What we need:
Our goal is to find the pattern of autophagy associated genes in various stages and types of cancers so that it can be helpful to deduce the dual role of autophagy in cancer. Moreover, we want to know the impact of these transcriptomic and mutational patterns on functional/mechanistic consequences of core-autophagy interactions as well as their individual (protein) functions in relation to LIRs interactions. In this context, we would be interested in collaborations with colleagues who have cellular models for different cancer types that we can use for validation of our hypothesis on changes in expression for the autophagy machinery. In addition, we seek for collaborations with experts in biochemistry for protein-protein interactions to validate the predictions on the effect of cancer mutations in the binding between LC3 proteins and LIR peptides.

Acknowledgment:
The project was supported by Danmarks Grundforskningsfond (DNRF125) and a Carlsberg Foundation Distinguished Fellowship (CF18-0314). Moreover, the project has been supported by a Netaji Subhash ICAR international fellowship, Govt. of India to MK to work in EP group. The calculations described in this paper were performed using the DeiC National Life Science Supercomputer Computerome at DTU (Denmark), and DECI-PRACE 14th HPC Grant for calculations on Archer (UK).

References:
1.Yun et al, 2018. Int J Mol Sci . 19(11): 3466.
2.Fas et al, 2019,bioRxiv, https://doi.org/10.1101/635284
3.Kumar et al, 2019, https://doi.org/10.1101/702522

Abstract no. 20

Systematic deletions reveal functional roles of the loops in *Trichoderma reesei* Cel7A

Nanna Røjel[1], Corinna Schiano di Cola[1], Kenneth Jensen[2], Jeppe Kari[1], Trine Holst Sørensen[1], Kim Borch[2] and Peter Westh[3]

[1]Roskilde University, Denmark
[2]Novozymes A/S, Denmark
[3]Department of Biotechnology and Biomedicine, Technical University of Denmark, Denmark

Glycoside hydrolase family 7 (GH7) cellulases are some of the most efficient degraders of cellulose, making them particularly relevant for industries seeking to produce renewable fuels from lignocellulosic biomass. The secretome of the cellulolytic model fungus *Trichoderma reesei* contains two GH7s, termed TrCel7A and TrCel7B. Despite having high structural and sequence similarities, the two enzymes are functionally quite different. TrCel7A is an exolytic, processive cellobiohydrolase (CBH), with high activity on crystalline cellulose, whereas TrCel7B is an endoglucanase (EG) with a preference for more amorphous cellulose. At the structural level, these functional differences are usually ascribed to the flexible loops that cover the substrate-binding areas. TrCel7A has an extensive tunnel created by eight peripheral loops, and the absence of four of these loops in TrCel7B makes its catalytic domain a more open cleft. To investigate the structure–function relationships of these loops, here we produced and kinetically characterized several variants in which four loops unique to TrCel7A were individually deleted to resemble the arrangement in the TrCel7B structure. Analysis of a range of kinetic parameters consistently indicated that the B2 loop, covering the substrate-binding subsites -3 and -4 in TrCel7A, was a key determinant for the difference in CBH- or EG-like behavior between TrCel7A and TrCel7B. Conversely, the B3 and B4 loops, located closer to the catalytic site in TrCel7A, were less important for these activities. We surmise that these results could be useful both in further mechanistic investigations and for guiding engineering efforts of this industrially important enzyme family.

Abstract no. 21

Shining light on split fluorescent protein fitness landscapes using peptide array-based deep substitutional scanning

Oana N. Antonescu, Kristoffer E. Johansson, Jakob R. Winther

Linderstrøm-Lang Centre for Protein Science, University of Copenhagen

Numerous split fluorescent proteins (split-FPs) have been developed in the last two decades. This is due to their wide applicability as biosensors for protein-protein interactions, genetically encoded tags for protein detection and localization, as well as fusion partners in super-resolution microscopy [1]. Still, a quantitative platform for engineering the affinity and photo-physical properties of split-FP fragments is lacking.

Here we present the first attempt to map the thermodynamic and functional landscape of a split-FP, by using peptide arrays. Peptide array technology is a powerful and highly cost-effective alternative to the biological synthesis, genotyping and assaying typically used in protein fitness mappings. We have demonstrated this applicability by synthesizing 183,612 green fluorescent protein strand 10 peptides on a single high-density array and assaying them for split-FP complementation, by fluorescence imaging.

Results show that the assay is capable of comprehensively mapping the fitness of the split-FP interaction at amino-acid resolution, with high precision and robustness. By saturation substitution of the wild-type strand, we have identified the peptide segments and residues that are essential for split-FP fluorescent complementation. The array fluorescence intensities correlate with the affinity between the split-FP fragments and the spectral properties of the reconstituted FPs in solution.

We have established and validated a novel platform for engineering the energetics and brightness of split-FPs, in high throughput and with rapid turnover. The assay does not require individual handling of clones at any step and avoids the laborious steps of DNA mutational scanning, thus we propose it as a versatile and powerful alternative to the currently available cell-based methodologies.

References:

1. Romei, M.G. and S.G. Boxer, Split Green Fluorescent Proteins: Scope, Limitations, and Outlook. Annu Rev Biophys, 2019. 48: p. 19-44

Abstract no. 22

Identification and Structural Analysis of Alginate Oligosaccharide Binding Sites on β-lactoglobulin

Sanaullah Khan[1], Mikkel Madsen[1], Emil G. P. Stender[1], Johnny Birch[1], Christian Kjeldsen[2] Lau D. Nielsen[3], Richard Ipsen[4], Günther H. J. Peters[2], Jens Ø. Duus[2], Kristoffer Almdal[2], Peter Westh[1], Birthe B. Kragelund[3], Pernille Harris[2], Finn L. Aachmann[5], Birte Svensson[1]

[1] Department of Biotechnology and Biomedicine, Technical University of Denmark (DTU), DK-2800 Kgs. Lyngby, Denmark,
[2] Department of Chemistry, DTU, DK-2800 Kgs. Lyngby, Denmark,
[3] Department of Biology, University of Copenhagen, DK-2200 Copenhagen, Denmark,
[4]Department of Food Science, University of Copenhagen, DK-1958, Frederiksberg C, Denmark,
[5]Department of Biotechnology and Food Science, NOBIPOL, Norwegian University of Science and Technology, 7491 Trondheim, Norway.

Brief description of research area

Polysaccharide-protein complexes are key in food texture formation and typical of certain dairy (yogurt and cheese) and other fermented foods. Previously, we characterized the interaction of two alginates (ALG) of different mannuronic acid (M)/guluronic acid (G) ratios with β-lactoglobulin (BLG) using dynamic light scattering and isothermal titration calorimetry (ITC)[1].

What we know

To gain molecular level insights on structural determinants in ALG-BLG complexes, alginate oligosaccharides (AOSs) with degree of polymerization of 3 (DP3) were generated from ALG using a *Sphingomonas sp*. lyase. The AOSs structures were determined by ^{1}H and ^{13}C NMR spectroscopy and binding sites were identified on recombinant BLG using both chemical shift perturbation of backbone resonance peaks in the ^{1}H,^{15}N-HSQC NMR spectrum at pH 2.65 and 4.0 and X-ray crystallography at pH 3.0. The structure of the BLG dimer in complex with AOSs at pH 3.0 and 1.75 Å resolution showed a bound D-glucuronate residue, while two other binding sites on the BLG for AOSs were identified at pH 2.65 and pH 4.0 by NMR chemical shift perturbation. ITC of the AOSs gave K_D around 1 mM. The importance of the AOS composition and size was further analyzed with 9 different AOSs of DP 4-6 and defined M, G and alternating M/G content at pH 2.65 and 4.0. Interaction was also measured for technologically relevant high molecular weight ALGs (260-300 kDa) with three different M/G ratios (1.82, 1.1 and 0.55). This study can provide a basis for rational engineering of food texturizing complexes of BLG with alginate and other food hydrocolloids.

What we need

Since our aim is to characterize the interactions between alginate/AOSs and BLGA, we need to determine binding sites on BLGA for AOSs using crystallography and HDX-MS approach.

Acknowledgments

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Novo Nordisk Foundation to the project WPAC, and 3 1/3 PhD fellowships from the Technical University of Denmark.

References

[1] E. G. P. Stender, S. Khan, R. Ipsen, F. Madsen, P. Hägglund, M. Abou Hachem, K. Almdal, P. Westh, B. Svensson, *Food Hydrocoll.* **2018**, 75, 157-163.

Abstract no. 23

Further Structural Studies of the Lytic Polysaccharide Monooxygenases *Ao*AA13

Sebastian Jannick Muderspach, Tobias Tandrup, Kristian E. H. Frandsen, Jens-Christian Poulsen, Leila Lo Leggio

University of Copenhagen, Department of Chemistry, Universitetsparken 5, DK-2100 KBH Ø, Denmark

Brief description of research area:
We are mainly specialized in structure determination of carbohydrate active enzymes by X-ray crystallography.

What we know:
I have been working on the Lytic Polysaccharide Monooxygenases (LPMO) AoAA13 and several endo-beta-1,4-galactanases. Endo-beta-1,4-galactanases are enzymes capable of degrading galactan by hydrolysis. I have tested the degradation profiles of the galactanases from different origins using a high-performance anion exchange chromatography instrument. Furthermore, variants were constructed, tested and compared to determine the effect on the degradation profile.

LPMOs are copper containing enzymes capable of degrading recalcitrant polysaccharides. I have attempted to elucidate the differences in coordination at the active site between the two oxidation states of the catalytically active copper (Cu(I) and Cu(II)). This has been done by reducing the effective dose obtained by the crystal using helical data collection or synchrotron serial crystallography. Binding of soluble substrates for the *Ao*AA13 have also been studied using nano differential scanning fluorimetry.

What we need:
We would like to expand our knowledge on techniques capable of determining the crystallinity of powered polysaccharides, or techniques otherwise capable of proving activity of LPMO’s on crystalline substrates. Techniques capable of elucidating binding between the enzyme and insoluble substrates would also be of interest for me.

Abstract no. 24

Evolutionary pressure on substrate binding in heterogeneous catalysis

- Functional roles of a highly conserved asparagine residue in a loop of the processive cellulase Cel7A

Silke Flindt Badino†*, Trine Holst Sørensen ‡, Nanna Røjel†*, Kim Borch ‡ & Peter Westh†

†Section for Protein Chemistry & Enzyme Technology, DTU Bioengineering, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark
‡Novozymes A/S, DK-2800, Kgs. Lyngby, Denmark
*Department of Science and Environment, Roskilde University, DK-4000 Roskilde, Denmark

Fungal cellobiohydrolases (CBHs) depolymerize microcrystalline cellulose. Their elongated active site is shaped by several loops, which cover the cellulose strand. The size and composition of the peripheral loops have been identified as essential for the distinctive enzymatic mechanism of CBHs, and this has generated considerable interest in functional roles of the loops in this industrially important group of enzymes. However, many questions regarding the molecular underpinnings of loop function remain open. In the current work, we address this through biochemical characterization of mutants of the CBH Cel7A from *Trichoderma reesei*. Specifically, we produced a site saturation library for a highly conserved asparagine residue (N200) positioned in the so-called B2 loop. We used the biochemical data to identify the functional importance and hence elucidate the adaptive mechanism that underlies its conservation. Conventional Michaelis-Menten parameters did not reveal any particular role of N200. Thus, compared to the wild type, the maximal turnover was higher in over half of the N200X mutants and changes in the specificity constant were small. Steady-state measurements under enzyme excess (so-called inverse Michaelis-Menten analysis), on the other hand, uncovered that N200 played a key role for the enzyme's ability to attack a wide range of sites on the cellulose surface. We surmise that evolutionary pressure towards this property could be common among interfacially active enzymes.

What we know: Variant production, expression, purification, kinetic characterization, rate limiting step, structure-function relation

What we need: Simulations, bioinformatics, imaging

Abstract no. 25

Hybrid degradomics: interactivity of matrix metalloproteinases and the surrounding degradome at the wound edge

S. Savickas[1], E. Schoof [1], U.a.d. Keller [1, 2]

[1]*Technical University of Denmark, Lyngby, Denmark*
[2] *Institute of Molecular Health Science, Zurich, Switzerland*

Matrix metalloproteinases (MMPs) contribute to skin homeostasis, cutaneous wound repair, and skin carcinogenesis. Thereby, they do not only remodel the extracellular matrix, but they also play pivotal roles in immune cell recruitment, angiogenesis, and epithelial cell proliferation. At the wound edge, MMPs are secreted by migrating keratinocytes and fibroblasts and form an interdependent zymogen activation network. In this project, we apply a novel hybrid degradomics approach to map this MMP activation network and its interconnections on the molecular, cellular and tissue level.

By using recombinant mouse proMMPs 2, 3, 7, 8, 9, 10, and 13 we have developed parallel reaction monitoring (PRM) assays for detection of these proteases in complex biological matrices. These assays uniquely discriminate latent and active MMPs that allow monitoring zymogen removal. We applied our newly established technique to assess pairwise co-activation of MMPs and generated a first model of interconnected MMP activation. Furthermore, we have developed a strategy combining the sensitivity of Parallel Reaction Monitoring and the scalable nature of data independent acquisition to observe and quantify surrounding proteins in murine skin.

Harnessing the power of hybrid degradomics, we have built a framework for the detection of endogenous MMP activity and their surrounding proteome to study their interdependent activation in cell secretomes from keratinocytes and fibroblasts as well as in mouse wound skin. Ultimately, the delineation of the MMP activation network by hybrid proteomics will elucidate how its components temporally and spatially shape the proteome at epithelial-mesenchymal interfaces in conditions of controlled and uncontrolled cell proliferation.

Abstract no. 26

LypidDyn: A computational microscope to scrutinize membrane properties at the organelle-level

S.Scrima[1], E. Corcelle-Termau[2], Maeda K[2], S. Tooze[3], M. Tiberti[1], M. Jäättelä[2], M. Lambrughi[1], E. Papaleo[1,4]

[1]Computational Biology Laboratory, Center for Autophagy, Recycling and Disease, Danish Cancer Society Research Center, Copenhagen, Denmark
[2]Cell Death and Metabolism Unit, Center for Autophagy, Recycling and Disease, Danish Cancer Society Research Center, Copenhagen, Denmark
[3]The Francis Crick Institute, 1 Midland Road, London, NW1 1AT, U.K
[4]Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

The role of lipids and their metabolic pathways is among the most intriguing and promising research fields in cancer development and progression. In comparison to our extensive knowledge of gene expression or protein networks, our understanding of lipid organization is somewhat lagging behind. Nevertheless, data have elucidated the decisive role of lipids in cellular functions, from the constitution of plasma/organelles membranes to second messengers of signal transduction and regulation of proteins activity [1-2].

In this context, organellar lipidomics is rapidly advancing, in contrast with traditional lipidomic assays. Traditional methods usually lead to the loss of information regarding the subcellular distribution of lipid classes and the molecular identity of individual species of lipids. Increasing our knowledge in this field is crucial since alterations in the lipidome of cellular organelles can have a tremendous impact on membrane properties and related functions [3], resulting in different pathological conditions, from neurodegenerative diseases to cancer [4,5].

What we know:
Our group at the Danish Cancer Society Research Center has been developing an integrative framework to bridge data from single organelle lipidomics to in-silico membrane modelling and investigate their molecular properties, by all-atom and coarse-grained molecular dynamics simulations. To this purpose, we have built an automated computational pipeline, implemented in Python 3, LypidDyn, for the accurate analysis of the structural properties and dynamics of lipid bilayers simulations.

The framework is divided into four independent modules that can be run separately, depending on the user choice. Each performs a different type of analysis that is also able to account for embedded proteins into the membrane. LipidDyn has been developed to include parameters that can be used to validate simulation ensembles against experimental data [6].

One module estimates the thickness of the membrane (usually defined as the distance between phosphorus atoms in two leaflets) and the area per lipid (the surface of the cross-section of the cylindrical hydrocarbon part of the lipid), providing information about the fluidity of the system. A

second module computes 2D density maps, i.e., a visual representation of how the density of the membrane changes, on both the leaflets constituting the bilayer, giving insight on the system phase. The third module investigates the diffusion motions of the system. It extracts all the coordinates of each lipid residue of the system and defines “maps” that can be used to explore specific lipid clusters. Ultimately a fourth module estimates the deuterium order parameter, a measure for the orientational mobility of the bonds between the carbon and hydrogen atom of the acyl chain of the lipid, used for estimating the overall order of the membrane and details of the conformations that the atoms in the lipid tails adopt.

This computational platform has currently employed to study, how different compositions in sphingolipids affects the structural and dynamical properties of organelle-like membrane models, in collaboration with our colleagues in the Unit of Cell Death and Metabolism [7]

What we need:
Molecular dynamics simulations are a powerful tool to examine the properties of membrane systems, it is possible to explore properties and dynamics of membrane models on atomic-scale details, as the accurate determination of such properties experimentally is difficult. On the other hand, the predictive value of any MD simulation depends on the accuracy of the underlying model that describes interatomic interactions (force fields), thus it needs to be carefully integrated with experimental data. Indeed, we are seeking novel collaborations with experts in biophysical techniques employed to characterize the properties of lipid membrane (NMR spectroscopy or neutron scattering), as well as experts/developers of novel computational tools of analysis for bilayer simulations.

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References
[1] R. W. Gross and X. Han, Chem. Biol. 18.3, 284–291 (2011).
[2] Ackerman, D.; Simon, M. C. Trends Cell Biol. 2014, 24 (8), 472−8
[3] Klose et al. Curr Opin Cell Biol. 2013 25(4):406-13
[4] R. M. Adibhatla et al., Future lipidol., 2(4), 403–422 (2007).
[5] C. Corbet and O. Feron, Curr. Opin. Clin. Nutr. Metab. Care, 20(4), 254–260 (2017).
[6] David Poger, et al Biochimica et Biophysica Acta (BBA) - Biomembranes,1858(7),1556-1565, (2016)
[7] E. Corcelle-Termeau et al., Autophagy, 12(5), 833–49 (2016).

Abstract no. 27

A flexible loop of a starch-active AA13 LPMO

Sonja Kunstmann[1], Günther H.J. Peters[2], Maher Abou Hachem[1]

[1]Technical University of Denmark Department of Biotechnology and Biomedicine,Kgs. Lyngby, Denmark.
[2]Technical University of Denmark Department of Chemistry, Kgs. Lyngby, Denmark.

E-mail: rusku@dtu.dk

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Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent monooxygenases, which utilize hydrogen peroxide or molecular oxygen and extrinsic electron sources to oxidize glycosidic bonds of polysaccharides at the C1 or C4 position. LPMOs from auxiliary activity family AA13 in CAZy have been shown to be active on starch [1]. Starch-active LPMOs possess a shallow binding site to accommodate the helically organized amylose or amylopectin polymers of starch in contrast to cellulose-active counterparts with a flatter architecture [2]. In order to access the supramolecular insoluble starch, LPMOs of AA13 frequently occur as modular enzymes joint to a starch binding module from the carbohydrate binding module family 20 (CBM20) [3]. Proteomic analysis of fungal cultures growing on starch emphasized the abundance of both the isolated LPMO13 and the modular LPMO13-CBM20 in the starch secretomes [4]. Notably, the details of the mode of action of LPMO13 and their interaction with starch remain unknown.

We performed molecular dynamics simulations on LPMO13 based on the only structurally characterized LPMO to explore the dynamics and substrate recognition by this enzyme to further progress the structural understanding of these starch-active enzymes.

References:

[1] Vu V.V.; Beeson W.T.; Span E.A.; Farquhar E.R.; Marletta M.A. PNAS 2014, 111, 13822–13827.
[2] Lo Leggio L.; Simmons T.J.; Poulsen J.N.; Frandsen K.E.H.; Hemswort G.R.; Stringer M.A., von Freisleben P.; Tovborg M.; Johansen K.S.; De Maria L.; Harris P.V.; Soong CL.; Dupree P.; Tryfona T.; Lenfant N.; Henrissat B.; Davies G.J.; Walton P.H. Nature Communications 2015, 6, 5961.
[3] Nekiunaite L.; Isaksen T.; Vaaje-Kolstad G.; Abou Hachem M. FEBS Letters 2016, 590, 2737–2747.
[4] Nekiunaite L.; Arntzen M.Ø.; Svensson B. Vaaje-Kolstad G.; Abou Hachem M. Biotechnol Biofuels 2016, 9, 187.

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Abstract no. 28

Rate-limiting step and substrate accessibility of cellobiohydrolase Cel6A from *Trichoderma reesei*

Stefan Jarl Christensen[1], Jeppe Kari[1], Silke Flindt Badino[1], Kim Borch[2] and Peter Westh[3]

[1]Roskilde University, INM, Research Unit for Functional Biomaterials, 1 Universitetsvej, DK-4000, Roskilde, Denmark
[2]Novozymes A/S, krogshøjvej 36, DK-2880, Bagsværd, Denmark
[3]Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark

Brief description of research area:
The Cellobiohydrolase Cel6A is an important component of enzyme cocktails used for industrial conversion of biomass polymers into soluble carbohydrates (saccharification), which is an essential step in upcoming biorefineries. However, the kinetics of this glycoside hydrolase (GH) acting on its natural, insoluble substrate remains sparsely investigated. We have studied Cel6A from *Trichoderma reesei* (*Tr*Cel6A) with respect to pre-steady-state and steady-state kinetics, both on microcrystalline (Avicel) and amorphous (RAC) cellulose. We found that slow dissociation was limiting for the steady-state rate, and we suggest that this leads to an accumulation of inactive enzymes in front of irregularities (obstacles) on the cellulose surface. The turnover of the catalytic cycle (each hydrolytic event in a processive sweep) was too fast to be fully resolved, but a minimum value of ~20 s^{-1} could be established. This is among the highest values reported hitherto for cellulases. We conclude that *Tr*Cel7A, is a catalytically efficient enzyme with a poor capability of attacking a broad range of structurally distinct sites on the cellulose surface. This is an important observation for future design of cellulase variants with higher potential in industrial applications.

What we know: Pre-steady-state and steady-state kinetics of interfacial enzymes (incl. cellulases), variant production, expression, purification.

What we need: Simulations, bioinformatics (phylogenetic analysis and subfamily identification of cellulases), imaging

Abstract no. 29

The unlocked potential of venom gland transcriptomics, droplet microfluidics, and alternative protein scaffolds

Thomas Fryer[1] and Timothy P Jenkins[2,3]

[1]Department of Biochemistry, University of Cambridge, Cambridge CB3 0ES, UK
[2]Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, UK
[3]Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark

T.jenkins1993@gmail.com, tf275@cam.ac.uk

Snakebite envenoming has for a long time been an overlooked problem, despite the hundreds of thousands of deaths and disabilities caused by this disease each year. Fortunately, technological advances, alongside an increase in public funding, are now presenting a new horizon for snakebite therapeutics.

Hence, researchers are exploring novel approaches towards treating snakebite envenomations, such as the deployment of monoclonal immunoglobulin G antibodies (IgG). However, whilst there have been promising results in the development of IgG based recombinant antivenoms, non-antibody protein scaffolds provide an intriguing alternative or addition to the development of next-generation antivenoms.

Indeed, such scaffolds are likely to overcome some of the limitations of IgGs, while retaining several of their benefits. Particularly, designed ankyrin repeat proteins (DARPins) and nanobodies carry significant promise due to their impressive safety profile in humans (established through rigorous clinical testing), high thermostability (potentially making cold chain distribution to remote locations redundant), low cost of manufacture, and high engineerability. Furthermore, recent developments in phage display and droplet microfluidics have the potential to enable ultra-high-throughput discovery and development of antitoxins, while venom gland transcriptomics could hold the key to global key-toxin identification.

If we harness the combined potential of these scaffolds and of high-throughput and cutting edge-technologies, we could provide a novel, cutting edge, and high-tech solution for global envenomings that is still affordable to those most in need.

Abstract no. 30

Laccase-catalyzed oxidation of lignin induces production of H_2O_2

Valentina Perna[1], Anne S. Meyer[1], Jesper Holck[1], Lindsay D. Eltis[2], Vincent G.H. Eijsink[3] and Jane Wittrup Agger[1]

[1]Section for Protein Chemistry and Enzyme Technology, Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby 2800, Denmark
[2]The University of British Columbia, Life Sciences Institute, Departments of Microbiology and Biochemistry, 2350 Health Sciences Mall, CA-Vancouver
[3]Norwegian University of Life Sciences, Faculty of Chemistry, Chr. M. Falsensvei 1, NO-1430 Ås

Brief description of research area:
Laccase (benzenediol: oxygen oxidoreductases; EC 1.10.3.2; AA1) are blue multi-copper oxidoreductase enzymes produced ubiquitously by fungi, plants and bacteria (and even humans) currently receiving particular attention because they catalyse the oxidation of phenolic compounds similar to subunits in lignin using only molecular oxygen as final electron acceptor. Laccases can oxidize lignin, initially producing a phenoxy radical. Lignin can stabilize these reactive radicals due to its heterogeneous structure, but eventually they dissipate by uncharacterized mechanisms.

We have shown that it possible to monitor the radical concentration during laccase oxidation of lignin using Electron Paramagnetic Resonance (EPR) spectroscopy and that subsequently some of these radicals lead to formation of H_2O_2, likely via reaction between lignin radicals and O_2. We have also shown that the generated H_2O_2 activated lytic polysaccharide monooxygenases (LPMOs). These observations are new and our results reveal a potentially critical connection between conversion of lignin and polysaccharides suggesting that laccases could generate H_2O_2 for other lignocellulose-depolymerizing enzymes such as lignin peroxidases and LPMOs.

What we know:
We know how to monitor the ongoing formation of radicals during laccase catalyzed oxidation of lignin. Some of these radicals are converted into H_2O_2 which is high enough to activate LPMOs oxidation of chitin and cellulose.

What we need:
We need methods to address the changing that lignin is undergoing after laccase oxidation in order to understand how the lignin structure is left after laccase reaction.

Abstract no. 31

The assembly of β- and κ-casein mixed micelles: thermodynamic and structural considerations.

W. Wijaya[1], S. Khan[1], P. Westh[1], M. S. Møller[1], M. Madsen[1], R. Ipsen[2], B. Svensson[1]

[1]Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, Denmark
[2]Department of Food Science, University of Copenhagen, Frederiksberg, Denmark

Brief description of research area:
In this work, we observe casein-casein interactions using calorimetry and light scattering techniques. β- and κ-casein were used as model of caseins. According to previous studies, β-casein might contribute to form internal core of the micelles and κ-casein might adsorb at the surface of the micelles. However, this existing theoretical knowledge is still highly debatable. In addition, probing the interactions between these two micellar proteins could be useful for tuning the sub-micron micelles which are useful for several bio-related applications.

What we know:
In this moment, we are studying the molecular interactions of β- and κ-casein using a calorimetric approach where we found that the interactions of these two proteins altered the micelles assembly of the individual protein. The structural studies using light scattering techniques were also used to explain the structural properties of mixed β- and κ-casein micelles. In the presence of κ-casein, the addition of β-casein as a function of concentration did not change the size of κ-casein by dynamic light scattering analysis, indicating a new type of micelles was formed. The SAXS analysis also confirmed that the ellipsoidal structures of the mixed casein micelles were formed with a reduced size if κ-casein was presence.

What we need:
Since the assembly of mixed casein micelles is still a black box, we are eager to be joined by new expertise on protein molecular labeling for imaging analysis to have a better understanding, which sites on caseins sites are interacting. Moreover, through this molecular labeling, we hope to monitor the assembly in real time and also to quantify the interactions. Other suggestions regarding characterization techniques of mixed micelles interactions are very welcome.

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Abstract no. 32

Integrative Ensemble Modeling of a Large Membrane Protein Complex Using Diverse and Ambiguous Information

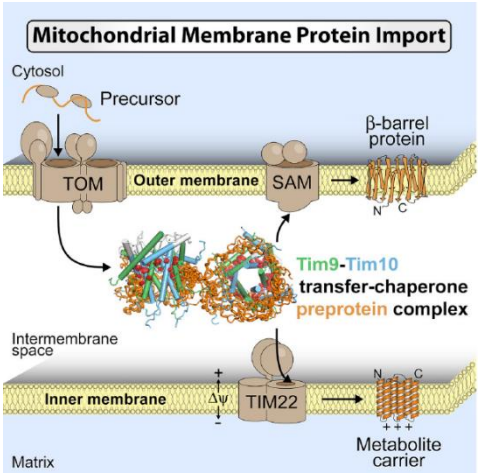
Yong Wang[1], Katharina Weinhäupl[2], Paul Schanda[2], and Kresten Lindorff-Larsen[1]

[1]*Structural Biology and NMR Laboratory, Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, DK-2200 Copenhagen N, Denmark*
[2]*Institut de Biologie Structurale, University of Grenoble Alpes, CEA, CNRS, 38042 Grenoble, France*

Mitochondria contain approximately 1200 different proteins, 99% of which are synthesized on cytosolic ribosomes and need to be delivered into the right destination through the intermembrane space by transport machineries, such as the TIM chaperone. Currently, the mechanistic and structural details of how the TIM chaperone binds to these mitochondrial proteins remain elusive.

To gain structural insight into the binding and chaperone mechanisms, we focused on the complex of the TIM9/10 chaperone and the mitochondrial GDP/GTP carrier membrane protein (Ggc1). Such complexes are difficult to study because they consist of a transiently formed, dynamic complex between two folded proteins and a membrane protein that should be solubilized and bound by the chaperone. X-ray crystallography has revealed the core structure of the free chaperone protein, but because of the dynamic nature and large size (~1400 amino acids) of the complex its structural features have remained elusive.

Using an integrative approach that combines biochemical assays, NMR spectroscopy and SAXS it was, however, able to obtain detailed but ambiguous information on the structures of the complex. In particular, the experiments showed that the complex consists of two well-structured (TIM9)3/(TIM10)3 hexamers bound to a mostly disordered Ggc1.



In this work, we developed a protocol to integrate such heterogeneous experimental data with a coarse grained molecular model to provide a description of the conformational ensemble of the TIM9/10-Ggc1 complex. In particular, we used a hybrid structure-based model (to describe the intra-molecular interactions within the folded chaperone), an NMR-derived contact potential for chaperone-client interactions and a knowledge-based potential (to describe the inter-molecular interactions between the chaperones and chaperone-client interactions). We used molecular dynamics (MD) simulations to sample the conformational landscape of the complex, and the resulting coarse-grained

conformational ensemble was subsequently converted into all-atom resolution and refined using a Bayesian/Maximum Entropy reweighting approach using the SAXS data.

This allows us to generate a weighted ensemble in agreement with experimental measurement. Such integrative structural modeling method is useful to generate a structural ensemble of large and dynamic proteins in a both efficient and reliable way.

References:

Katharina Weinhäupl, Caroline Lindau, Audrey Hessel, Yong Wang, Conny Schütze, Tobias Jores, Laura Melchionda, Birgit Schönfisch, Hubert Kalbacher, Beate Bersch, Doron Rapaport, Martha Brennich, Kresten Lindorff-Larsen, Nils Wiedemann* and Paul Schanda*. Structural Basis of Membrane Protein Chaperoning Through the Mitochondrial Intermembrane Space. Cell, 175, 1365- 1379, (2018)

The mechanism of recognition and binding between a BH3-only protein and Bcl-xL using all-atom molecular simulations

Dionisio Sánchez Cabrera[1], Matteo Lambrughi[1] , Elena Papaleo[1,2]

1 Computational Biology Laboratory, Center for Autophagy, Recycling and Disease, Danish Cancer Society Research Center, Copenhagen, Denmark

2 Translational Disease Systems Biology, Faculty of Health and Medical Sciences, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark

Brief description of the research area

Apoptosis, a specific form of programmed cell death, is an important regulatory process inside the cell. Mechanisms of apoptosis are often deregulated in cancer, which makes it an interesting and relevant topic of research to tackle with different approaches, also at the molecular and biophysical levels. More specifically, it has been proposed that interactions between a class of proteins known as *BH3-only*, a subset of the anti-apoptotic Bcl-2 family of proteins, and other Bcl-2 proteins are crucial to modulate apoptosis [1]. An interesting case study is an interaction of the Bcl-2 protein Bcl-xL with the BH3-only protein Puma, a competitor of the tumor suppressor p53 for Bcl-xL sequestration. The binding of Puma to the BclxL BH3-binding pocket allosterically causes p53 release from Bcl-xL and triggers a cascade of events leading to apoptosis through mitochondrial outer membrane permeabilization [2] Conformational changes induced by the binding of Puma to Bcl-XL occur in two of the Bcl-xL helices (α2 and α3) in the immediate proximity of the hydrophobic groove where the BH3-only peptides bind [2]. NMR data suggest that this region of interest seems to adopt a more disordered conformation in the Puma-bound form. However, a detailed description at the atom level on the intrinsic propensity of disordered conformations in the Puma-unbound state of the Bcl-xL protein is missing. Moreover, the intermolecular interactions between Puma and Bcl-xL which can drive these conformational changes remain elusive.

What we know: Our group at the Danish Cancer Society Research Center is focusing on the study of the conformational ensemble of Bcl-xL alone and in complex with Puma and other BH3-only proteins using molecular dynamics simulations integrated to available NMR data. In line with this purpose, we have run a first exploration with different all-atoms Molecular Dynamics (MD) simulations in explicit solvent with different force fields. We then compare the performance of both force fields (CHARMM22* and AMBER99SB-disp [3]) by quantifying the level of agreement of available experimental chemical shift data with the predicted chemical shifts of the simulated ensembles. Assessment of the quality of protein models using experimental data is crucial to guide force field selection and gain an accurate dynamics description. The first analyses of the unbiased MD trajectories that we collected revealed important aspects concerning the dynamics of Bcl-xL, along with a first insight into the structural changes undergone by Bcl-xL upon binding to the BH3 of Puma. However, classical MD simulations are limited in the sampling of rare

conformational changes occurring on long timescales and in the capability of observing multiple events of transitions between different conformational states of proteins, which are needed for statistical analyses. Metadynamics, an enhanced sampling technique that can be applied to MD simulations [4], can be used to explore regions of the conformational space hardly accessible in the time scale of unbiased simulations and thus offer a more complete description of protein dynamics. For the Bcl-xL protein, this will mean a quantitative assessment of the unfolding of our region of interest when the protein is alone, a better knowledge of the extent to which the BH3-only peptide is responsible for such events in the bound-state. Biased trajectories along collective variables that account for the folding/unfolding at the level of our region of interest are currently under production. Complementary approaches such as Protein Network Analysis or mutation analyses have also been ongoing to identify differential interactions that are associated with the folded/unfolded states of the BH3 binding groove of Bcl-xL, to suggest collective variable to assess in the metadynamics framework.

What we need: In our research on Bcl-xL and Puma mechanisms, we look for collaborations with groups in experimental biophysics to design experiments on mutant variants of the two proteins to validate our hypothesis on the mechanism of the key residues for the binding-induced unfolding, along with to assess the intrinsic propensity of disorder in the free state of the Bcl-xL protein. Different biophysical techniques could be useful in this context, including single-molecule based spectroscopies and NMR.

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References

[1] 3] Kalkavan, Halime, and Douglas R. Green. "MOMP, cell suicide as a BCL-2 family business." *Cell death and differentiation* 25.1 (2018): 46.

[2] Follis, Ariele Viacava, et al. "PUMA binding induces partial unfolding within BCL-xL to disrupt p53 binding and promote apoptosis." *Nature chemical biology* 9.3 (2013): 163.

[3] Robustelli, Paul, Stefano Piana, and David E. Shaw. "Developing a molecular dynamics force field for both folded and disordered protein states." *Proceedings of the National Academy of Sciences* 115.21 (2018): E4758-E4766.

[4] Barducci, Alessandro, Massimiliano Bonomi, and Michele Parrinello. "Metadynamics." *Wiley Interdisciplinary Reviews: Computational Molecular Science* 1.5 (2011): 826-843.

List of Participants

First name	Surname	Title	Affiliation	Email	Poster no.
Agnes	Beenfeldt Petersen	BSc.		agnesbeenfeldt@gmail.com	
Ákos	Kovács	Professor	DTU Bioengineering	atkovacs@dtu.dk	
Aleksander	Haack	Student	DTU	s153292@student.dtu.dk	
Alexander	Büll	Prof.	DTU Bioengineering	alebu@dtu.dk	
Alexander	Hebbelstrup	MSc		Hebbelstrup@live.com	
Allan	Nørgaard	Science Manager	Novozymes	alnq@novozymes.com	
Amelie	Stein	Professor	University of Copenhagen	amelie.stein@bio.ku.dk	1
Ana	Mendes	Researcher	DTU Food	anac@food.dtu.dk	
Anders	Malmendal	Dr	Roskilde University	amalm@ruc.dk	
Andreas	Laustsen	Associate Professor	DTU Bioengineering	ahola@bio.dtu.dk	
Andreas	Prestel	PhD	University of Copenhagen	andreas.prestel@bio.ku.dk	
Anna	Dragos	PhD	DTU Bioengineering	adragos@dtu.dk	
Anne	Blicher	Laborant	University of Copenhagen	abl@ign.ku.dk	2
Anne S	Meyer	Professor	DTU Bioengineering	asme@dtu.dk	
Annemarie	Matthes	Dr	University of Copenhagen	annemarie.matthes@plen.ku.dk	

Bartlomiej	Kolaczkowski	MSc	DTU Bioengineering	bmakol@dtu.dk	3
Birgitte	Zeuner	Assistant Professor	DTU Bioengineering	bzeu@dtu.dk	4
Birte	Svensson	Professor	DTU Bioengineering	bis@bio.dtu.dk	
Bjarke Bak	Christensen	Head of Dept	DTU Bioengineering	bbch@dtu.dk	
Bodil	Kjeldgaard	PhD student	DTU Bioengineering	bkjeld@dtu.dk	
Casper	Wilkens	Protein artist	DTU Bioengineering	cwil@dtu.dk	
Catarina	Fernandes	PhD student	University of Copenhagen	catarina.fernandes@bio.ku.dk	
Cathrine	Larsen	MSc.student	University of Copenhagen	cathrine.larsen@bio.dtu.dk	
Cecilia	Romanò	Dr	DTU Chemistry	ceroma@kemi.dtu.dk	
Cecilia	Rossetti	Dr.	DTU Chemistry	cero@kemi.dtu.dk	
Cecilie	Søltoft	Laboratoriekoordinator	University of Copenhagen	cecilie.soltoft@bio.ku.dk	
Celeste	Hackney	Ms	University of Copenhagen	celeste.hackney@bio.ku.dk	
Céline	Galvagnion	Dr	University of Copenhagen	celine.galvagnion@sund.ku.dk	
Chengxin	Li	PhD student	DTU Chemistry	cheli@kemi.dtu.dk	
Christian	Nielsen	PhD student	DTU Bioengineering	chrfn@dtu.dk	
Christian	Dybdahl Andersen	PhD student	Aarhus University	chda@inano.au.dk	5
Corinna	Schiano di Cola	PhD student	DTU Bioengineering	codico@dtu.dk	6

Danai	Sismani	Ms		danaesismani@gmail.com	
Daniel	Bavnhøj	Student	DTU	s193126@win.dtu.dk	
Daniel	Otzen	Professor	Aarhus University	dao@inano.au.dk	
David	Teze	Postdoc	DTU Bioengineering	davtez@dtu.dk	
Deniss	Petrovs	PhD Student	DTU Bioengineering	denpet@dtu.dk	7
Ditte	Welner	Group Leader	DTU Biosustain	diwel@biosustain.dtu.dk	
Eduardo	Moreno	Mr		emoreno.dk@gmail.com	
Elnaz Z.	Fallahasghari	Master student		elnaz.fallah@yahoo.com	
Emil G. P.	Stender	Postdoc	DTU Bioengineering	emigst@bio.dtu.dk	
Emilie	Müller	PhD student	DTU Bioengineering	emmul@dtu.dk	
Fatemeh	Ashari Ghomi	Postdoc	DTU Biosustain	fatgho@biosustain.dtu.dk	
Federico	Casanova	Postdoc	DTU Food	feca@food.dtu.dk	
Flemming	Jessen	Senior Researcher	DTU Food	fjes@food.dtu.dk	
Folmer	Fredslund	Staff scientist	DTU Biosustain	folf@biostustain.dtu.dk	
Franziska	Zosel	Senior Research Scientist	Novo Nordisk	fszo@novonordisk.com	
Giuseppe	Dionisio	Researcher	Aarhus University	giuseppe.dionisio@mbg.au.dk	
Günther	Peters	Professor	DTU Chemistry	ghp@kemi.dtu.dk	
Gustavo	Avelar Molina	PhD student	DTU Bioengineering	gumol@dtu.dk	

Hao	Jiang	Postdoc	DTU Chemistry	haoji@kemi.dtu.dk	
Heike	Rösner	Assistant professor	University of Copenhagen	heike.rosner@bric.ku.dk	
Henrik	Daver	Dr.	University of Copenhagen	henrik.daver@sund.ku.dk	
Ian Max	Møller	Professor Emeritus	Aarhus University	ian.max.moller@mbg.au.dk	
Ida	Vedel	PhD student	DTU Chemistry	idaved@kemi.dtu.dk	
Irene	Shim	Associate professor	DTU Chemistry	shim@kemi.dtu.dk	
Jacob Hertz	Martinsen	Academic Staff	University of Copenhagen	jacob.martinsen@bio.ku.dk	
Jakob	Winther	Professor	University of Copenhagen	jrwinther@bio.ku.dk	
Jan	Nowak	PhD student	Aarhus University	jsn@inano.au.dk	8
Jan	Vester	Research Scientist, PhD	Novozymes	jkjv@novozymes.com	
Jane	Wittrup Agger	PhD	DTU Bioengineering	jaag@dtu.dk	
Javier	Linares-Pastén	Dr.	Lund University	javier.linares_pasten @biotek.lu.se	
Jennifer Virginia	Roche	Postdoc	University of Copenhagen	jennifer.roche@bio.ku.dk	
Jenny	Arnling Bååth	Postdoc	DTU Bioengineering	jkere@dtu.dk	
Jens	Duus	Professor	DTU Chemistry	jduus@kemi.dtu.dk	
Jens	Eklöf	Science Manager	Novozymes	jeeq@novozymes.com	
Jens Erik	Nielsen	Director	Novozymes	jein@novozymes.com	

Jens Preben	Morth	Professor	DTU Bioengineering	premo@dtu.dk	
Jeppe	Tams	Science Manager	Novozymes	jwta@novozymes.com	
Jeppe	Kari	Postdoc	DTU Bioengineering	jepka@dtu.dk	
Jesper	Vind	Senior science manager	Novozymes	jvi@novozymes.com	
Jesper	Holck	Senior researcher	DTU Bioengineering	jesho@dtu.dk	
Jesper	Salomon	Scientist	Novozymes	Jrsx@novozymes.com	
Jialei	Zhang	Recent graduate		jialeizhang95@gmail.com	
Jingwen	Qiu	PhD student	DTU Bioengineering	jiqiu@dtu.dk	
Johan Ørskov	Ipsen	Mr		johanoerskov@gmail.com	
Johanna	Tiemann			johanna.tiemann@gmail.com	
Johanna	Koivisto	PhD candidate	University of Copenhagen	johanna.koivisto@bio.ku.dk	9
Julia	Tanasi	Laboratory Technician	DTU Bioengineering	jutata@dtu.dk	
Julie	Bomholt	PhD		jbo@aquaporin.dk	
Julie	Leth	PhD		julie.maja@finsenlab.dk	
Kaj Frank	Jensen	Professor emeritus		kajfrankjensen@gmail.com	
Karoline	Leth	MSc student	University of Copenhagen	qcm713@alumni.ku.dk	
Kasper	Tidemand	Research Scientist	Novozymes	kadt@novozymes.com	

Katja	Johansen	Professor	University of Copenhagen	ksj@ign.ku.dk	
Katrine	Landbo		DTU Bioengineering	akrl@dtu.dk	
Kay	Schaller	Mr.	DTU Bioengineering	kaysc@dtu.dk	10
Kenneth	Jensen	Science Manager	Novozymes	khjn@novozymes.com	
Kirk	Schnorr	Senior Science Manager	Novozymes	kksc@novozymes.com	
Konstantinos	Kalogeropoulos		DTU Bioengineering	konka@dtu.dk	11
Kresten	Lindorff-Larsen	Professor	University of Copenhagen	lindorff@bio.ku.dk	
Kristian	Barrett	PhD Student	DTU Bioengineering	kbaka@dtu.dk	12
Kristian	Krogh		Novozymes	kbk@novozymes.com	
Kristian Kølby	Kristensen	PhD student		kristian.kristensen@finsenlab.dk	
Kristoffer	Johansson	Assistant professor	University of Copenhagen	kristoffer.johansson@bio.ku.dk	
Kristoffer	Almdal	Professor	DTU Chemistry	kral@dtu.dk	
Larissa	Staack	Industrial PhD student	DTU Bioengineering	larissa@dtu.dk	
Lea	Rasmussen	Research assistant	DTU Bioengineering	learas@dtu.dk	
Line Abildgaard	Ryberg	PhD		linearyberg@gmail.com	13
Lisa	Merklinger	Ms	DTU Bioengineering	lismer@dtu.dk	
Mads	Clausen	Professor	DTU Chemistry	mhc@kemi.dtu.dk	

Magnus	Haraldson Høie	PhD		magnus.hhoie@gmail.com	
Maher	Abou Hachem	Prof MSO	DTU Bioengineering	maha@bio.dtu.dk	
Manish K.	Tiwari	Assistant Professor	University of Copenhagen	tiwari@chem.ku.dk	
Maria	Leth	phd	DTU Bioengineering	malole@bio.dtu.dk	
Maria Dalgaard	Mikkelsen	PhD	DTU Bioengineering	mdami@dtu.dk	14
Marie Sofie	Møller	Assistant professor	DTU Bioengineering	msmo@dtu.dk	15
Marlene	Vuillemin	Postdoc	DTU Bioengineering	mavu@dtu.dk	
Martin	Grønbæk-Thygesen	PhD student		martinthygesen@rocketmail.com	
Martin	Willemoës	Assoc. Prof.	University of Copenhagen	willemoes@bio.ku.dk	
Martina	Weibull	Student	University of Copenhagen	bdc728@alumni.ku.dk	
Marzanna	Due	Lab technician	University of Copenhagen	mdue@plen.ku.dk	
Matteo	Lambrughi			matteolambrughi@gmail.com	16
Matteo	Tiberti	Dr		tiberti@cancer.dk	17
Mette Errebo	Rønne	PhD student	DTU Bioengineering	meero@dtu.dk	
Michael	Ploug	Group Leader		m-ploug@finsenlab.dk	
Michael	Pichler	Mr	DTU Bioengineering	mijap@dtu.dk	
Mikkel	Madsen	PhD student	DTU Bioengineering	mikmads@dtu.dk	18

Mohamed Attia Shaaban	Mahmoud	Prof.		mshaaba@gmail.com	
Mona	Ghafari	mrs		ghafari.sabah@gmail.com	
Mukesh	Kumar	Mr.		mkum@cancer.dk	19
Mustapha Carab	Ahmed	Mr.	University of Copenhagen	Mustapha.carab@bio.ku.dk	
Nanna	Rhein-Knudsen	Postdoc	DTU Bioengineering	nark@dtu.dk	
Nanna	Røjel	Postdoc	Roskilde University	nnsr@ruc.dk	20
Nicole	Trier	Postdoc	SSI	nhp@ssi.dk	
Ninfa Rangel	Pedersen	Dr		Ninfa.rangel.pedersen@gmail.com	
Oana-Nicoleta	Antonescu	PhD Fellow	University of Copenhagen	oana.antonescu@bio.ku.dk	21
Paolo	Marcatili	Associate Professor	DTU Bioinformatics	pamar@dtu.dk	
Patrick	Raunft	BSc		patrick.raunft@web.de	
Pengfei	Tian	Senior Data Scientist	Novozymes	pti@novozymes.com	
Pernille	Harris	Professor	DTU Chemistry	ph@kemi.dtu.dk	
Peter	Heegaard	Professor	DTU Bioengineering	pmhh@dtu.dk	
Peter	Westh	Professor	DTU Bioengineering	petwe@dtu.dk	
Peter Sørensen	Millard	Postdoc	University of Copenhagen	peter.millard@bio.ku.dk	

Prafull	Gandhi	Principal Scientist	Novo Nordisk	pgan@novonordisk.com	
Preben	Morth	Professor	DTU Bioengineering	premo@dtu.dk	
Rebekka	Jensen	Student	DTU	s164131@student.dtu.dk	
Roland	Pache	Dr.	Novozymes	roap@novozymes.com	
Sanaullah	Khan	Dr.	DTU Bioengineering	sank@dtu.dk	22
Sanne	Schoffelen	Dr.	DTU Biosustain	sansch@biosustain.dtu.dk	
Sara Ester	Viejo	Ms		sara_sther@hotmail.com	
Sarah	Hedberg	Dr	DTU Chemistry	shmhe@kemi.dtu.dk	
Sebastian	Meier	Dr	DTU Chemistry	semei@kemi.dtu.dk	
Sebastian	Muderspach	PhD student	University of Copenhagen	sjm@chem.ku.dk	23
Shang	Lin	Ph.D	DTU Bioengineering	shanli@dtu.dk	
Silke	Badino	Post doc	DTU Bioengineering	sillbad@dtu.dk	24
Simonas	Savickas	PhD student	DTU Bioengineering	simsav@dtu.dk	25
Simone	Scrima	Research Assistant		simonescrima@gmail.com	26
Sine	Larsen	Professor emerita	University of Copenhagen	sine@chem.ku.dk	
Sonja	Kunstmann	PhD	DTU Bioengineering	rusku@dtu.dk	27
Sophie	Heiden Laugesen	Research assistant	University of Copenhagen	sophie.laugesen@bio.ku.dk	
Steen	Brewer	Msc. Biotechnology	DTU	s182833@win.dtu.dk	
Stefan	Kol	Dr.		stef.kol@gmail.com	

Stefan Jarl	Christensen	Post.Doc	Roskilde University	sjarlc@ruc.dk	28
Steffen	Goletz	Professor	DTU Bioengineering	sgoletz@dtu.dk	
Stjepan Kresimir	Kracun	Dr.	Chr. Hansen	DKSTKK@chr-hansen.com	
Suk Kyu	Ko	PhD student	DTU Chemistry	sukkyk@kemi.dtu.dk	
Susan	Preus	Research assistant	DTU Bioengineering	suspre@dtu.dk	
Søren	Brander	Postdoc	University of Copenhagen	sbd@ign.ku.dk	
Søren	Vedel	Data scientist	Novo Nordisk	sqve@novonordisk.com	
Tiia	Kittilä	Dr.	DTU Biosustain	tiiaki@biosustain.dtu.dk	
Timothy	Jenkins	Dr	DTU Bioengineering	tpaje@dtu.dk	29
Trenton	Stewart	Mr.		trentonstewart36@gmail.com	
Tune	Wulff	Protein analyst	DTU Biosustain	tuwu@biosustain.dtu.dk	
Ulrich	auf dem Keller	Professor MSO	DTU Bioengineering	uadk@dtu.dk	
Valentina	Perna	Industrial Postdoc	DTU Bioengineering	valper@dtu.dk	30
Vinoth	Wigneswaran	Postdoc	DTU Bioengineering	vinwi@dtu.dk	
Wahyu	Wijaya	Dr.	DTU Bioengineering	wahwi@dtu.dk	31
Willy	Bjørklund	Area Manager LSMS Omics Nordics	Thermo Fisher	Willy.bjorklund@thermofisher.com	
Xinxin	Xiao	Dr.	DTU Chemistry	xixiao@kemi.dtu.dk	

Yicen	Lin	PhD student	DTU Bioengineering	yicenli@dtu.dk	
Yong	Wang	Postdoc		wyongciac@gmail.com	32
Yulian	Gavrilov	postdoc	University of Copenhagen	yulian.gavrilov@bio.ku.dk	
Zhenwei	Ni	PhD	DTU Chemistry	zhni@kemi.dtu.dk	

Speakers

First name	Surname	Title	Affiliation	Email
Anne	Imberty	Research Director	Centre de Recherches sur les Macromolécules Végétales (CERMAV), Grenoble, France	Anne.Imberty@cermav.cnrs.fr
Antoine	Loquet	Group Leader	Institut Européen de Chimie et Biologie (IECB) Université de Bordeaux, France	a.loquet@iecb.u-bordeaux.fr
Bernard	Henrissat	Research Director	Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille University, Luminy, France	Bernard.Henrissat@afmb.univ-mrs.fr
Emma	Master	Professor	Department of Chemical Engineering and Applied Chemistry, University of Toronto, Canada	emma.master@utoronto.ca
Frank	Sobott	Professor	The Astbury Centre for Structural Molecular Biology, University of Leeds, United Kingdom	f.sobott@leeds.ac.uk
Frido	Welker	Postdoctoral Researcher	Globe Institute, University of Copenhagen, Denmark	frido.welker@bio.ku.dk
Johan	Åqvist	Professor	Department of Molecular and Cellular Biology, Uppsala University, Sweden	Johan.Aqvist@icm.uu.se

John	Christodoulou	Professor	Biological NMR Spectroscopy, University College London, United Kingdom	j.christodoulou@ucl.ac.uk
Julia	Shifman	Principal Investigator	Alexander Silberman Institute of Life Sciences, Hebrew University Jerusalem, Israel	jshifman@mail.huji.ac.il
Thomas	Barends	Group Leader	Department of Biomolecular Mechanisms, Max Planck Institute for Medical Research, Heidelberg, Germany	thomas.barends@mpimf-heidelberg.mpg.de

PROTEIN.DTU Task Force

First name	Surname	Title	Affiliation	Email
Birte	Svensson	Professor	DTU Bioengineering	bis@bio.dtu.dk
Flemming	Jessen	Senior Researcher	DTU Food	fjes@food.dtu.dk
Günther H. J.	Peters	Professor	DTU Chemistry	ghp@kemi.dtu.dk
Jenny	Emnéus	Professor	DTU Bioengineering	jemn@dtu.dk
Kristoffer	Almdal	Professor	DTU Chemistry	kral@kemi.dtu.dk
Maher Abou	Hachem	Professor	DTU Bioengineering	maha@bio.dtu.dk
Pernille	Harris	Professor	DTU Chemistry	ph@kemi.dtu.dk
Peter M. H.	Heegaard	Professor	DTU Bioengineering	pmhh@dtu.dk