16th Protein.DTU Workshop

Abstract Book

Networking for Young Researchers in Protein Science

Monday, May 22, 2017
Lecture Hall 54, building 208 – Kemitorvet, 2800 Kgs. Lyngby

Contents

Program

08:30 – 09:00	**Arrival & Breakfast**
09:00 – 09:10	**Welcome** Chair: Birte Svensson (Professor, DTU Bioengineering)
09:10 – 09:40	**Quantum Biochemistry** **Jan Halberg Jensen** (Professor, KU Chemistry) Chair: Günther Peters (Associate Professor, DTU Chemistry)
09:40 – 10:10	**Recombinant therapeutic glycoproteins: Improving the productivity in Chinese hamster ovary cells** **Henning Gram Hansen** (Postdoc, DTU Biosustain) Chair: Peter Heegaard (Professor, DTU Vet)
10:10 – 10:40	**Understanding the evolution and specificity of lysine acetylation signaling** **Chuna R. Choudary** (Professor, NNF Center for Protein Research, KU) Chair: Flemming Jessen (Senior Scientist, DTU Food)
10:40 – 11:10	**Coffee Break**
11:10 – 12:20	**FLASH PRESENTATIONS - 4 min each** Chairs: Kristoffer Almdal (Professor, DTU Nanotech) Charlotte B. Madsen (Senior Scientist, DTU Food) Pernille Harris (Asso. Professor, DTU Chemistry)
12:20 – 13:30	**Lunch and poster viewing**
13:30 – 14:00	**Regulation of bacterial toxin activity through protein higher-order structure and symmetry** **Ditlev E. Brodersen** (Associate Professor, AU, Dept. of Molecular Biology and Genetics) Chair: Pernille Harris (Associate Professor, DTU Chemistry)
14:00 – 14:30	**Protein structure on the verge of collapse - or Why are ancestral proteins more stable?** **Jakob R. Winther** (Professor, KU, Faculty of Science, Linderstrøm-Lang Center) Chair: Kristoffer Almdal (Professor, DTU Nanotech)
14:30 – 15:00	**You're not alone – What can you do for your gut microbes, and what can they do for you?** **Tine Rask Licht** (Professor, DTU Food) Chair: Maher Abou Hachem (Associate Professor, DTU Bioengineering)
15:00 – 16:30	**POSTER SESSION, coffee and "time for a beer"** Chairs: Maher Abou Hachem (Asso. Professor, Bioengineering) Flemming Jessen (Senior Scientist, DTU Food) Peter Heegaard (Professor, DTU Vet)

List of Poster Abstracts

Characterisation of non-histone N-ε acetylation in probiotics

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Brief description of research area

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

What we know

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

What we need

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

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

Evolution-based approach for converting glycosidases into transglycosidases

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Brief description of research area: We aim at synthesizing oligosaccharides and/or glycoconjugates with glycoside hydrolases (GH). These enzymes usually hydrolyse instead of synthesize the desired oligosaccharides, so we need to mutate them into "transglycosidases". To achieve this goal, we designed an evolutionary-based approach, which allow us to select the residues the most likely to influence the balance between hydrolysis and synthesis, as well as finding mutations that can be transferred from one enzyme to another within a GH family. This approached have proven to be successful on two GH families (GH1 and GH36) that act through a glycosyl-enzyme intermediate, allowing us to produce both α- and β-linked oligosaccharides. We currently work on applying it to a glycoside hydrolase that presents a substrate-assisted mechanism (GH20).

What we know: We have a working knowledge of basic molecular biology and protein production & characterization, as well as basic organic synthesis, enzyme kinetics, and various analytical tools.

What we need: We would like to learn or be helped by people with skills in programming, bioinformatics and molecular dynamics simulation (particularly QM/MM).

Development of glycosyltransferases for sustainable denim production

Ditte Welner[1], Tammy Hsu[2], Birte Svensson[1], John Dueber[2]
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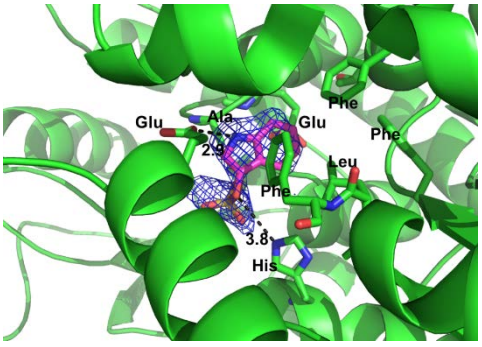
Blue jeans are the western world's favorite garment and its iconic, fading color comes from indigo dye. More than 40,000 tons of indigo is produced annually with the predominant purpose of dyeing denim[1]. Conventional chemical indigo production and dyeing is ecologically devastating and encompasses significant occupational hazards. It is energy-intensive, uses toxic and carcinogenic substances and high concentrations of alkali. It releases toxic wastewater that corrodes the piping of the wastewater treatment facilities. Consequently, little indigo production and dyeing are done in countries with rigorous environmental regulations. Although eco-friendly biotech indigo produced in engineered bacteria has been known for more than a decade[2], it is not cost-competitive. One reason is the cost and environmental impact of the extraction process of the insoluble indigo from the bacterial cells.

We are developing a strategy widely used in nature, namely to solubilize indigo as the stable glycoside indican by glycosylating the indigo precursor indoxyl. Indican can in turn be readily hydrolyzed to indoxyl by β-glucosidase, and oxidized to indigo. We have identified two plant glycosyltransferases capable of glycosylating indoxyl using UDP-glucose as the donor substrate. When the corresponding genes are transferred into *E. coli* that have been engineered to produce indoxyl, we can recover 2-5 grams of indican per liter of culture.

We have solved the crystal structure of these glycosyltransferase enzymes and characterized the indoxyl binding mode by co-crystallization with indoxyl sulfate. These enzymes adopt the canonical GT-B fold consisting of two Rossmann domains connected by a long linker and forming a catalytic cleft, where an elongated substrate pocket accommodates the nucleotide sugar donor and the indoxyl acceptor[3]. The acceptor binding site is located in one end of this elongated pocket that is predominantly made up by residues in the N-terminal Rossmann domain. Here, the heterocyclic portion of indoxyl sulfate is accommodated by a handful of perpendicular hydrophobic stacking interactions, and the ring nitrogen is forming a hydrogen bond to a conserved glutamate (figure). A loop region that are only found in certain subclasses in the glycosyltransferase classification[4] may play an important role in catalytic efficiency and specificity. This structure will form the basis for enzyme engineering to manipulate specificity, efficiency and stability in the future.



1. Steingruber (2004) Ullmann's Encyclopedia of Industrial Chemistry
2. Berry *et al.* (2002) J Ind Microbiol Biotechnol 28:127-33
3. Lairson et al (2008) Annu Rev Biochem 77:521–55
4. Mackenzie et al (2005) Pharmacogenet Genomics 15: 677-85

Meta-proteomic characterization of protein regulation in multi-species biofilm

Zacharias Brimnes Visby Damholt[1, 4], Jakob Herschend[2], Andrea Marion Marquard[3], Birte Svenson[4], Søren J. Sørensen[2], Per Häglund[1] and Mette Burmølle[2]

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[2] Section for Microbiology, Department of Biology, University of Copenhagen, Denmark

[3] Section for Immunology and Vaccinology, National Veterinary Institute, Technical University of Denmark, Denmark

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Abstract

The on-going global interest in microbial biofilms continues to show the prevalence of biofilms in nature and their impact on our society. Most biofilms in natural environments are highly complex microbial communities shaped by interspecies interactions and growth conditions. Such interactions modify the functionality of the multispecies biofilm, as compared to biofilms formed by the individual species, and may create synergistic communities. In order to improve our understanding of these molecular interactions and their downstream protein signaling, a meta-proteomic approach was applied to describe the interactions in a highly synergistic multispecies biofilm consortium.

Single species and co-cultures were grown in a Drip Flow Reactor system to allow the biofilm to develop at the air-liquid interface under low shear stress conditions. Development of techniques for efficient protein extraction from the biofilms was first established. Hereafter the various biofilms generated were compared through proteomic profiling.

Unique protein profiles were observed in the multispecies biofilm as compared to those generated by the individual species. The unique profiles indicated changed functionality of all involved species in the multi-species biofilm.

Additionally, unique protein profiles were found at the individual time points of the biofilm succession, revealing changes in protein expression patterns for all the involved strains over time.

This meta-proteomics study demonstrates a new way to describe the functionality of multi-species biofilms and contributes to a deeper understanding of the underlying molecular background of interspecies interactions.

Relevant activities:

In my PhD project I also work with identification of post-translational modification, such as S-nitrosylation and tyrosine nitration. Our work on these modifications both involves clinical proteomics and development of blood-based biomarkers as well as studying the regulation of the S- nitrosylation in cell signaling. I therefore have a broad interest and knowledge in mass spectrometry based proteomics.

Therefore I have a special interest in a conference on post-translational modifications in cell signaling and new advances in the techniques involved in detecting them.

Protein biochemistry at NNF Biosustain: quantification of Erythropoietin and alpha-1-antitrypsin

Stefan Kol[1], Thomas Beuchert Kallehauge[1], Henning Gram Hansen[1], Simon Adema[2], Helle Munck Petersen[1], Pim Hermans[2], Helene Faustrup Kildegaard[1], Gyun Min Lee[1,3], and Bjørn Voldborg[1]
(1) NNF Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby, Denmark. (2) BAC BV, Thermo Fisher Scientific, Naarden, The Netherlands. (3) Department of Biological Sciences, KAIST, Daejeon, Republic of Korea.

Abstract
The CHO Cell Line Engineering department is addressing the need to rapidly obtain high yields and quality of biopharmaceuticals produced in optimized CHO cells through genome-scale-based methodologies. Our main goals are to develop genome editing technologies, generate omics data, genome-scale in silico models, and high-throughput screening methods to improve CHO cell engineering efforts and facilitate identification of factors that control biopharmaceutical protein production. Based on Section input, we characterize our engineered CHO cell lines with respect to growth, morphology, and titer of a set of model proteins.
Erythropoietin (EPO) and alpha-1 antitrypsin (A1AT) quantification during cell line selection and bioreactor cultivation has traditionally been performed with ELISA. As these techniques suffer from several drawbacks, we developed a novel quantification assay. A camelid single domain antibody fragment was evaluated as a capturing antibody in a label-free biolayer interferometry-based quantification assay. Human recombinant EPO and A1AT can be specifically detected in Chinese hamster ovary cell supernatants. These methods enable rapid and robust quantification in a high-throughput setting. We compared the obtained EPO titer with ELISA and found that they were in good agreement. In addition, an in-depth analysis was performed of different quantification methods for A1AT.

What we know
Heterologous protein production, protein purification, protein analysis, biolayer interferometry, titer analysis

What we need
CZA, CIEF, Amino terminal sequence analysis, Circular dichroism spectroscopy

Exopolysaccharide-induced aggregation of β-lactoglobulin studied by solution scattering and analytical ultracentrifugation

Sanaullah Khan[1], Johnny Birch[1], Pernille Harris[2], Marie-Rose Van Calsteren[3], Richard Ipsen[4], Günther H.J. Peters[2], Birte Svensson[1] and Kristoffer Almdal[5],

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Brief description of research area

Bacterial exopolysaccharides (EPSs), commercially important biopolymer, have recently attracted considerable attentions due to their improved physical and rheological properties in fermented milk products. Despite the fact that a very large number of bacterial exopolysaccharides have been reported, only a few have emerged as industrially important biopolymers. This is mainly because knowledge on their molecular structures and details on associative interactions with proteins is lacking. In the present study, we have characterized EPSs from various strains of lactic acid bacteria and their interactions with β-lactoglobulin.

What we know

By combining small-angle X-ray scattering (SAXS), dynamic light scattering (DLS) and analytical ultracentrifugation (AUC) in conjunction with molecular modeling, we have characterized heteroexopolysaccharides (HePS-1–HePS-4) from Lactic acid bacteria (LAB) and their interactions with β-lactoglobulin. We showed that these HePSs exhibited compact conformation in solution using SAXS and DLS combined with scattering modeling. Our SAXS, DLS and AUC data for these HePSs mixed with β-lactoglobulin showed that β-lactoglobulin forms large aggregates.

What we need

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

References

Khan, S., Birch, J., Harris, P., Van Calsteren, M-R., Ipsen, R., Peters, G. H. J., Svensson, B., Almdal, K. (2017). Revealing the compact structure of lactic acid bacterial heteroexopolysaccharides by SAXS and DLS. *Biomacromolecules*. **18**, 747–756.

Barley limit dextrinase and its endogenous inhibitor as model system for high affinity binding

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Brief description of research area:
Recently, crystal structures of starch debranching limit dextrinase (LD) from barley in complex with substrates and products as well as its endogenous inhibitor limit dextrinase inhibitor (LDI) have given new insight into starch degradation as well as regulation [1,2]. LDI is from a family of α-amylase and serine-protease inhibitors, the cereal-type inhibitors. It binds to LD with picomolar affinity, and we have evidence for interaction hotspots in the complex with LD. Furthermore, LDI has a very high thermostability (Tm=97.4°C) [2].

Proteinaceous enzyme inhibitors have been shown to not only regulate the activity, but also stabilize their target enzymes. The presented work focuses on design of inhibitors of a group of industrial enzymes, starch hydrolases. Inhibitors can be used for regulation of enzymes and recycling enzymes from industrial processes.

What we know:
The properties of LDI makes it an interesting starting point for design of binders for industrial processes. The approach combined advanced computational methods including the software Rosetta and experimental characterisation of inhibitor variants using e.g. surface plasmon resonance and yeast surface display. Initial results have shown that LDI is a stable backbone for design, as mutation of up to 10% of all amino acid residues did not reduce the thermostability of the protein. Through the design process we have identified LDI variants, which maintain inhibition activity towards LD, even though several interface residues have been changed, highlighting properties of high affinity protein-protein complexes.

What we need:
We have been constructing a single site saturation yeast surface display library including all interface residues of LDI. It has been analysed using fluorescence-activated cell sorting (FACS). We have now several datasets from next-generation sequencing, but we need someone, who know how to do the analysis of these data, i.e. in the end we would like to know which variants at amino acid level ends up in which pool, and by this be able to say something about influence on affinity.

[1] M.S. Møller, M.S. Windahl, L. Sim, M. Bøjstrup, M. Abou Hachem, O. Hindsgaul, M. Palcic, B. Svensson, A. Henriksen, J. Mol. Biol. 427(6) (2015) 1263–1277. [2] M.S. Møller, M.B. Vester-Christensen, J.M. Jensen, M. Abou Hachem, A. Henriksen, B. Svensson, J. Biol. Chem. 290(20) (2015) 12614–12629.

Acknowledgements:
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Detemir -Albumin Interactions studied by small-angle X-ray scattering and *in-silico* modelling:

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[2]Novozymes A/S, 2820 Bagsværd, Denmark. [3]CMC Assist Aps, 2500 Copenhagen, Denmark.

Brief description of research area:
Protein drugs are increasingly important in the pharmaceutical industry but, unfortunately, suffer from an inherent physical instability that limits manufacturability and formulation development. A strategy for stabilization of the drugs is non-covalent association to recombinant human serum albumin (rAlb). In this project, protein-protein interactions between rAlb and different protein drugs are investigated to understand the stabilization on a molecular level.

What we know:
We use an interdisciplinary approach to study protein-protein interactions. With dynamic light scattering, the stoichiometry of a protein complex is determined. With small-angle X-ray scattering (SAXS), attractive and repulsive inter-particle interactions are investigated, and a low-resolution bead model of the complex can be obtained. With molecular dynamics (MD) simulations, interactions between proteins are investigated on a molecular level. The set-up of the MD simulations are based on the low-resolution model obtained by SAXS.

What we need:
We are interested in supplementary techniques that do not require labelling of or mutations in the protein. We would like to learn more about:

- hydrogen-deuterium exchange (HDX) to determine the binding site in a protein complex,
- isothermal titration calorimetry (ITC) to investigate the energetics of binding,
- protein-protein docking using SAXS data as constraints (for example Rosetta),
- and guiding MD simulations with SAXS data (Gromacs implementation).

We are generally very interested in combining experiments with computational work, and we would like learn more about the opportunities.

Identification of an array of proteases expressed by *Amycolatopsis keratiniphila* growing on pig bristles and nails

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[2] DTU Chemical Engineering, DTU Bioengineering, Department of Biotechnology and Biomedicine, Technical University of Denmark, Søltofts Plads, Building 227, DK 2800 Kgs. Lyngby, Denmark

[3] Protein and Immune Systems Biology, DTU Bioengineering, Technical University of Denmark, Building 301, DK 2800 Kgs. Lyngby, Denmark

Meat production from pigs is a resource heavy process as for example the pig feed often stems from soya beans grown in South America. Every part of the animal that is not used constitutes a protein food-chain lose, something that is not viable neither economically nor environmentally. The goal of this project is to better harness resources from slaughterhouse waste e.g. keratin rich pig bristles and nails through bacterial and fungal conversion. We have chosen to study the bacterial specie *Amycolatopsis keratiniphila*, which is known to degrade other keratin-rich substrates. However, little is known about the mode of degradation or the enzymes involved. Here we identify some of the possiple important key players that make *Amycolatopsis keratiniphila* capable of living of keratin-rich substrates as its only source of nitrogen and carbon.

Funding for the K2P project was partially paid for by “Innovation Fund Denmark”. This PhD stipend was partially paid for by DTU.

What we know: We do proteomics on the culture supernatant and purified protein fractions

What we don’t know/what we need: Protease active site titration with aza-peptides

Glycan utilization by the butyrate producing *Roseburia hominis* of the healthy gut microbiota

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The human gut microbiota (HGM), has a profound impact on human physiology and health [1]. A pivotal factor that modulates the composition of this community is preferential catabolism of dietary host-non-digestible glycans, which are fermented to various short chain fatty acids (SCFAs). Fecal transplants have unveiled the tremendous potential of manipulating the HGM composition in treatment of metabolic disorders. Insight into the metabolic specialization amongst the HGM, however, offers a more controlled and rational approach to develop future microbiota-based therapeutic interventions. Recently, butyrate producing bacteria of the HGM have attracted particular attention, owing to the paramount role of butyrate in regulating host cellular differentiation, apoptosis, gene expression and inflammatory responses [2]. Expectedly, levels of butyrate producers inversely correlate with inflammatory disorders e.g. Crohn disease and ulcerative colitis. Notably, the majority of these taxa are predicted to be nutritionally highly specialized based on their set of carbohydrate active enzymes (CAZymes) [3]. In contrast to the more intensely studied classical probiotic bacteria from *Lactobacillus* and *Bifidobacterium* [4,5], insight into glycan metabolism of butyrate producers as new probiotic candidates is limited.

The main objective of this research project is to bring molecular insight into the glycan uptake and degradation machinery of the butyrate producer *Roseburia hominis* suggested to define dysbiosis in patient with inflammatory ulcerative colitis disorders [6]. Accordingly, we aim to identify and characterize different CAZymes and transport proteins mediation glycan degradation and metabolism. Furthermore, it is aimed to identify competitiveness and the metabolic interactions of R. hominis with other members of the HGM.

What we know:
Growth under of strictly anaerobic conditions, protein production, different forms of protein analyses, protein-protein / protein-ligand interactions analyses

What we need:
Transcriptomics, proteomics, qPCR

[1] J. Clemente et al., Cell 148(6), (2012) 1258-1270
[2] P. Louis et al. FEMS Microbiology Letters 294(1), (2009) 1-8 [3] H. Harries et al. Microbial Genomics 2(2) (2016)
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[5] J.M Andersen et al., Proc. Nactl. Acad Sci. 108(43), (2011) 17785-17790
[6] K. Machiels et al., Gut 63(8), (2014) 1275-1283

Acknowledgements: This PhD project is funded by the Technical University of Denmark

Identification of cross-linked peptides in proteins subjected to photo-oxidation

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Brief description of research area:

The goal of this study was to develop a workflow aimed at identifying and characterizing cross-links in protein samples subjected to free radical oxidation using optimized ^{18}O isotopic labelling, different types of mass spectrometry acquisition workflows, and a designated database software tool.

What we know:

Selected proteins were cross-linked via a photo-oxidation process using Rose Bengal or incubation of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). Proteins were digested with trypsin in the presence of either ^{16}O water or ^{18}O water, mixed and analyzed using LC-MS/MS (Orbitrap Fusion, Thermo Fischer) using three acquisition methods. Data analysis was carried out both by manual spectral interpretation and by using different software including MaxQuant, GPMAW and MassAI. A *Lactococcus lactis* protein extract exposed to AAPH oxidation was also used in order to evaluate this approach for cross-linking identification in more complex samples.

By applying this workflow, different types of cross-linked peptides with undefined chemistry were identified, including tyrosine-tyrosine, tyrosine-tryptophan and tyrosine-lysine cross-links. The presence of these cross-links is indicated by the 8 Da shift in the MS spectra due to the ^{18}O labeling and also by the loss of 2 Da in the cross-linked peptide mass due to the nature of these particular cross-links.

Different fragmentation techniques were also evaluated, including HCD, ETD and EThcD. It was shown that EThcD fragmentation provided higher sequence coverage of cross-linked peptides, while HCD resulted in higher number of identified cross-links.

What we need:

The workflow here described is aimed at the identification of cross-links in samples subjected to free radical oxidation. Identifying cross-links with undefined chemistry is still however a very challenging task: ^{18}O labeling strategy is not always efficient and bioinformatics software for cross-links identification only work if one has an idea of the possible cross-linking chemistry. The ultimate goal is therefore to optimize and try to apply this workflow to more complex and clinically relevant samples, and possibly also discovering new types of cross-links.

The isoform-specific substrate inhibition mechanism of human tryptophan hydroxylase

Kasper D. Tidemand[1], Günther H. Peters[1], Pernille Harris[1], and Hans E. M. Christensen[1].
[1] Department of Chemistry, Technical University of Denmark.

Brief description of research area:
Tryptophan hydroxylase (TPH) catalyses the initial step in the synthesis of serotonin. Dysregulation of serotonin is associated with a variety of disorders such as depression and irritable bowel syndrome. TPH exists in two isoforms, where isoform 1 catalyses the synthesis of serotonin in the peripheral tissues and isoform 2 catalyses this step in the brain. The catalytic domains of the isoforms have a sequence identity of 81 % and their tertiary structures are therefore very similar. Despite the high sequence identity, differences in the kinetic parameters of the isoforms have been identified, i.e. only isoform 1 displays substrate tryptophan inhibition.

What we know:
Steady-state kinetic results show that the kinetic parameters of only isoform 1 are significantly changed upon mutations in a loop lining the active site. Mutations in the active site loop of isoform 1 results in an increase in the substrate inhibition constant, K_i, and therefore turnover rate. Mutations in the loop of isoform 2, to corresponding residues of isoform 1, results in an introduction of substrate inhibition. The change in the kinetic parameters are not caused by a change in stability or state of oligomerization, as analytical gel filtration and differential scanning fluorimetry have shown that the isoforms and all mutant variants are monodisperse and equally thermostable.

What we need:
How does the loop participate in substrate inhibition mechanism? How do the non-conserved residues in the loops ~20 Å away from the active site iron result in different kinetics of the isoforms?

Classification of Protein Therapeutics According SCOP Database

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What we know:

Classification of 3D structures is important for understanding the principles of protein structure and function. Protein therapeutics from FDA database were structurally classified according SCOP database. From our investigation it is clear that there must be more protein therapeutics to be discovered. The number of represented folds/classes is really small compared to the total number of folds listed in the SCOP database, which englobes 1431 different folds. Protein therapeutics only cover 30 of these corresponding to only 2,1%. It is possible to draft three most common folds: a.26, g.1 and b.1. a.26 is 4-helical cytokines fold that represents 13% of total protein therapeutics. g.1 is insulin like and b.1 is immunoglobulin-like beta-sandwich fold that correspond to 13% and 22% respectively. Membrane proteins have not been explored at all in terms of protein therapeutic development.

What we need:

Using all collected information related with existing protein therapeutics, we want to connect formulation conditions to structure and other physicochemical properties. If we can find some connection, it might be useful for formulation development of new protein therapeutics in the future.

Role of reactive oxygen species in photo-induced acceleration of lytic polysaccharide monooxygenase

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Brief description of research area

Lytic polysaccharide monooxygenases (LPMO) are cellulose oxidizing enzymes. The enzymes are especially interesting due to their ability to oxidize cellulose in crystalline form.
The activity of LPMO have recently been shown to increase in an electron donating system activated by light.(1) It has been debated if LPMOs recent discovered acceleration is linked to one or more of the reactive oxygen species produced as byproducts, or whether the activating effect is caused by direct electron transfer.(2)

What we know

By systematically scavenging reactive oxygen species and monitoring oxygen consumption in the LPMO/photosensitizer system no evidence has been found that reactive oxygen species is the cause of the activating effect. Results from this project points toward a direct electron transfer to LPMO as the most likely mechanism behind the observed acceleration of the enzymatic process.

What we need

Prove that direct electron transfer is the mechanism behind the light driven activity.

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Classical and Steered Molecular Dynamics Simulations to Study Cellobiohydrolase (Cel7A) - Cellulose Interactions

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Brief description of research area:

Enzymes that degrade cellulose to glucose by hydrolyzing the 1,4-β-D-glycosidic bonds are used in the industrial production of bioethanol. Currently, the reaction rate is approx. 1 hydrolysis per enzyme per second and slow compared to other enzymatic processes[1]. Because the enzymatic hydrolysis is one of the costliest steps in the production of bioethanol, an industrial interest exists for increasing the reaction rate further. The binding site in most cellulases can be described as a tunnel in the enzyme. The loops surrounding the binding tunnel are possible targets for engineering that can potentially modify the catalytic rate. We have used classical molecular dynamics (MD) simulations in combination with steered MD simulations to investigate the association / dissociation of cellulose to / from the binding pocket of cellulase and to map temporary interactions during these processes.

What we know:

The rate-limiting step for the overall catalytic reaction is believed to be the dissociation of cellulose from cellulase[2,3]. If dissociation is the rate-limiting step, changes making the dissociation faster should also increase the overall reaction rate. Therefore, by minimizing the energy barriers (decreasing the binding affinity) during the sliding dissociation motion of cellulose from the binding tunnel, the reaction rate should be increased. Quantitative Information about mobility and interactions have been identified using MD simulations and steered MD simulations. Prediction of changes in binding affinity has been estimated using the linear interaction energy method, calibrated to experimental data from binding isotherms. Experimental determination of enzyme kinetics (Michaelis-Menten) has been done for a variety of enzyme variants, to see if proposed mutations gave better enzymes.

What we need:

If dissociation is the rate-limiting step, then an energy barrier for the dissociation reaction must also exist. If the energy barrier can be identified, the amino acids interacting with the substrate at the peak of this barrier could be potential sites for site-directed mutagenesis to increase catalytic efficiency.

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Computational methods to investigate the role of sphingolipids in the regulation of structure and dynamics of membrane proteins and implications for cancer progression

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Sphingomyelin (SM) plays 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 such as the essential autophagic protein Atg9A, but the molecular details of the SM-protein interaction and its role in regulating functions are still elusive. A huge number of proteins from channels to receptors and transporters interact with lipids, but the structural effects still remain largely elusive. The comprehension of lipid- protein relationships is of primary importance since their alterations are associated with cancer, representing highly prospecting target mechanisms for novel treatments.

What we know: Abnormal levels of SM are associated with cancer and Niemann-Pick type A disease, causing autophagy defection by trapping Atg9A in recycling endosomes and subsequent failure of autophagic membranes to close and fuse with lysosomes, as recently demonstrated in Jäättela's group [1]. We are here using cutting-edge computational methods, like atomistic simulations, that will permit to investigate in molecular details the mechanisms of SM interaction with membrane proteins and rationalize results obtained with advanced cell biology, biochemistry, lipidomics and proteomics. We are studying the TM6 region of ATG9A and the following disordered tract (residues 470-523) that has been recently suggested to be important for protein trafficking and lipid interaction [2,3].

What we need: Molecular simulations are promising to describe the structural biology of lipid membranes and proteins [4,5]. Indeed while experiments generally permit to observe spatial and temporal average properties, molecular simulations can provide atomic level and detailed properties that are essential to rationalize them. We here show some computational methods to investigate the interaction between proteins and lipids and investigate their structural mechanisms that are crucial for understanding disease and cancer promoting mechanisms. In our young group at the Danish Cancer Society Research Center we are seeking to novel collaborators working with experimental or computational techniques to investigate lipids membrane and interactions with proteins.

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Exploring new dimensions of protein structure/sequence space

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Brief description of research area:
Protein engineering: design, structure and interaction, expression technologies
We wish to establish an experimental platform that can test and inform computational methods for protein design. For this purpose we use split-Green Fluorescent Protein and fragment complementation assays as tools to understand the relationship between protein sequence/structure and function.

What we know:

- Recombinant protein production in bacteria
- ✓ DNA cloning techniques (PCR, agarose gel electrophoresis)
- ✓ Protein expression and purification (SDS-PAGE, Western Blot, Affinity Chromatography, Size Exclusion Chromatography, Ion Exchange Chromatography)
- Protein interaction (UV-Vis and Fluorescence spectroscopy, ITC, DSC)
- Protein formulation and stability (using the above mentioned techniques)

What we need:

- Fluorescent microarray imaging
- Binding kinetics techniques (*e.g.* SPR)

Characterization of PIPPI on the Molecular Level Using *In-Silico* Simulation Techniques

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What we know:

Protein therapeutics have interested many pharmaceutical industries as they are often characterized by high specificity and potency with low toxicity. Several challenges confront pharmaceutical scientists involved in the development of protein therapeutics. Currently, a detailed molecular understanding of the effect of different physicochemical formulation conditions on the stability of proteins are sparse as molecular interactions are difficult to probe experimentally at the molecular level. Computational methods, such as molecular dynamics (MD) simulations, can provide insight on the single-molecule level. Our study currently involves investigating the effect of pH and ionic strength on the wild-type plectasin (known as antimicrobial defensins) and three variants of plectasin. Further, to identify hot spots essential for molecular interactions with the aim to correlate these results with experimentally generated data in the consortium. Thus, protein formulation projects can greatly benefit from computational methods that are able to identify potential hotspots for excipient-protein interactions and predict binding affinity of excipients to these regions.

What we need:

To study the effect of physiochemical conditions (pH, ionic strength) and excipients on protein stability and to identify protein-excipient interaction sites considering small, cysteine-rich peptide, plectasin. Using *in-silico* techniques, such as molecular docking and MD simulations, study is done to inspect the effect of excipients on protein stability and to identify excipient hot spot regions on proteins at varying pH and ionic strength.

Molecular details of alginate β-lactoglobulin interaction

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Brief description of research area:

Alginate is an anionic polysaccharide from brown algae that has many uses as a protein stabilizer, gelling agent, carrier and nanoparticle component. However, molecular details of alginate protein interaction are lagging behind. Alginate can be used to form nano-particles at pH below a proteins isoelectric point. Recently, it was hypothesized that the charge density on alginate may be important for interaction and particle formation (1). Furthermore, the charge density on the protein in the form of the protein polyelectrolyte binding site (charge patch) has been hypothesized to be very influential on the interaction strength (2). β-lactoglobulin, the predominant protein in whey, is monomeric below pH 3 and a dimer at pH 3.5 – 9 (3). We recently demonstrated that particle size, strength of interaction and alginate/β-lactoglobulin stoichiometry depend highly on pH (Stender et al., submitted). An alginate oligosaccharide (ALGOS), produced by treatment with alginate lyase, did not form particles, but still interacted with the protein. In this model system ^{15}N-HSQC NMR was used to identify β-lactoglobulin residues undergoing significant chemical shift perturbation caused by interaction with ALGOS. The protein charge patch responsible for the interaction was identified at pH 2.65 and 4.0 and while some affected residues differed between pH 2.65 to pH 4.0 several were the same (K75, K101). The findings support that the interactions between polyelectrolytes and proteins are not random. In the present system residues close to the dimerization interface of β-lactoglobulin were affected by interaction with ALGOS. This insight on anionic polysaccharide protein interaction can be useful in design of food nano-particles.

What we know:

Alginate oligosaccharides interacts with some of the same residues at pH 4 and pH 2.65. Differences are likely caused by deprotonation and surface charge.

What we need:

Homogenic alginate oligosaccharides of defined size without sodium to further the study.

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