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Flash Talk Abstracts

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Flash Talk Session A

FA1 -Identification of new putative ligands for the GnRH receptor

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Brain disorders like Alzheimer and brain cancer, are among the most common health problems of our society**.** Developing new therapeutic agents to treat such diseases is particularly challenging because of the general difficulties with carrying out *in vivo* studies, and the impossibility of accessing patient tissues. In addition, it may also be problematic to permeate the blood-brain barrier (BBB) in high enough concentration for a therapeutic effect. New and innovative therapeutic strategies precisely targeting the underlying disease mechanisms are needed. The Gonadotropin-Releasing Hormone (GnRH) and the Gonadotropin-Releasing Hormone Receptor (GnRH-R) are dysregulated in gliomas[1], Alzheimer[2], hormone-dependent diseases such as endometriosis, breast and prostate cancer, and therefore promising targets for therapeutic interventions[3]. Novel radiotracers addressing the human GnRH-R are needed to monitor, diagnose, and treat diseases related to the dysregulation. Computational aided drug design (CADD) is a key tool used for the design and development of new drugs, with the advantages to reduce cost and time in the drug discovery process.[4] Receptor-Based Virtual Screening is a CADD method that allow us to find molecules able to bind a specific target based on the 3D structure of the target. By using the crystal structure of the GnRH receptor and the chemical structure of known compounds able to bind, a virtual library of new putative ligands has been created and screened. Around fifteen compounds seem to be promising for further investigations and *in vitro* studies will be carried out to study the binding between the potential ligands and the receptor.

References

1. Lai, P. E. I. C., Chiu, T. E. D. H. & Huang, Y. E. N. T. A. Overexpression of BDNF and TrkB in human. *Anticancer Res.* **31**, 1265–1270 (2010).

2. Meethal, S. V., Smith, M. A., Bowen, R. L. & Atwood, C. S. The gonadotropin connection in Alzheimer's disease. *Endocrine* **26**, 317–325 (2005).

3. Marelli, Marina Montagnani, et al. "Novel insights into GnRH receptor activity: role in the control of human glioblastoma cell proliferation." *Oncology reports* 21.5 (2009): 1277-1282.

4. Cerqueira, N. M. F. S. A. *et al.* Receptor-based virtual screening protocol for drug discovery. *Arch. Biochem. Biophys.* **582**, 56–67 (2015).

FA2 - **The co-chaperone DNAJC12 binds to and stabilizes tyrosine hydroxylase**

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Tyrosine hydroxylase (TH) converts L-tyrosine to L-3,4-dihydroxyphenylalanine, which is the first and rate-limiting step in the synthesis of the catecholamines dopamine (DA), noradrenaline and adrenaline, which are essential neuromodulators for motor control, emotion, reward, biorhythms and learning. Mutations in *TH* can result in misfolded proteins with reduced stability, activity or altered substrate specificity that could eventually lead to the disease TH Deficiency (THD), which is associated with decreased DA levels, Dopa-responsive dystonia and infantile parkinsonism. Recently, TH was identified as one of the clients of the co-chaperone protein DNAJC12 and thus, its dysfunction may also cause symptoms of THD. As a heat shock protein 40 (Hsp40) protein, DNAJC12 is hypothesized to recognize and bind to client proteins and cooperate with Hsp70 to maintain client protein homeostasis. Apart from the J-domain that is characteristic of all DNAJ-proteins which is vital for its interaction with Hsp70s, DNAJC12 shares no homology with any other known protein and no other domains are predicted from its sequence. However, the heptapeptide "KFRNYEI" located at its very C-terminal end is conserved in all DNAJC12 homologues and has therefore been hypothesized to be involved in client binding. Our results show that DNAJC12 can be purified recombinantly as a monomer that binds with high affinity to TH. Through size exclusion chromatography coupled with multi-angle light scattering, we demonstrate that two DNAJC12s bind to tetrameric TH and through dynamic light scattering, we show that this interaction leads to the stabilization and aggregation delay of TH *in vitro*. The DNAJC12 mutant lacking the evolutionarily-conserved "KFRNYEI" peptide loses its ability to bind to client proteins and thus its ability to delay TH aggregation *in vitro*, supporting the hypothesis that this region has a vital role for the normal function of DNAJC12.

FA3- Investigation of hydroxymethylbilane synthase mutants and intermediates associated with acute intermittent porphyria

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Hydroxymethylbilane synthase (HMBS), the third enzyme in the haem biosynthesis, is associated with the metabolic dominantly inherited disorder acute intermittent porphyria (AIP). In AIP, mutations often result in a defective protein leading to loss of function or reduced functional protein. The reduced HMBS activity causes an accumulation of the haem building blocks δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG) in the body. These are toxic to the nervous system and clinically leads to acute life-threatening neuropsychiatric attacks.

HMBS catalyses the formation of the linear tetrapyrrole 1-hydroxymethylbilane (HMB) in a reaction where four consecutive PBG molecules are bound, creating the enzyme intermediate complexes ES, ES_2, ES_3 and ES_4 respectively, before releasing the final product HMB. In this work, we studied a selection of mutants in comparison with wild-type HMBS using a combination of native PAGE, anion-exchange chromatography, circular dichroism, and differential scanning fluorimetry techniques.

We have been able to show the distribution of intermediates corresponding to the different elongation steps using anion-exchange chromatography and native PAGE and we have confirmed the stages with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). We have also been able to show that the enzyme loses thermostability during its consecutive substrate-binding process and preliminary assays analysing reaction speed of wild-type HMBS using FT-ICR MS demonstrated that the reaction from the holoenzyme into the final product (E_{holo} to ES_4) is very quick.

The results from this work contribute to a better understanding of the molecular mechanisms of disease-causing mutants and aid in gaining a better understanding of the genotype-phenotype relationship for AIP. This alongside studies of the catalytic mechanisms of the mutants is vital to understand the kinetics of the enzyme and will aid the interpretation of mutational effect in disease-causing mutants, as well as how these can be modulated by drugs or pharmacological chaperones.

FA4 - Diversity in myoglobin function

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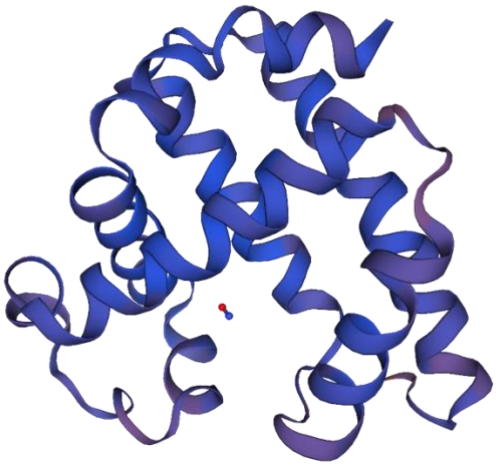
Myoglobin is a heme protein located in skeletal muscle in most vertebrates and functions in oxygen transport and storage. Myoglobin can also display complex redox chemistry involved in several important physiological process including reactive oxygen species (ROS) scavenging and nitric oxide metabolism.[1,2] Goldfish and the common carp are the only vertebrates known today that express two paralogs of myoglobin (Mb1 and Mb2). The paralogs are a result of a recent whole genome duplication.[3] Mb1 is expressed and functions in oxidative muscle tissue whereas Mb2 is only expressed in the brain. The tissue specific expression led to work showing that the two myoglobin isoforms functions differently.[4] In the present study, work is being done to gain structural and biophysical insight into the differences of these isozymes by comparing 3D structures, kinetics, and spectroscopic data. The reaction of myoglobin with hydrogen peroxide has been of particular interest. The presence of multiple redox states of myoglobin is observed during the reaction with H_2O_2.



Myoglobin 1 (Carassius auratus) structure prediction by SWISS-MODEL

[1] Eich, R.F. et al., Biochemistry, 35 (1996)
[2] Flögel, U. et al., FASEB J, 18 (2004)
[3] David, L. et al., Mol Biol Evol, 20 (2003)
[4] Helbo, S. et al., Am J Physiol Regul Integr Comp Physiol, 302 (2012)

FA5- Exploration of an alginate lyase targeting the biofilm in *Pseudomonas aeruginosa*

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Some strains of *Pseudomonas aeruginosa* change their morphology in the lungs of cystic fibrosis patients by overproducing alginate biofilm, becoming a mucoid variant that is more resistant to host defense and medical treatment. On the other hand, the bacterium does not only use alginate for biofilm production, as this negatively charged polysaccharide also is necessary for colonization and dispersion. *P. aeruginosa* possesses therefore a big variety of enzymes capable of synthesizing, modifying, and degrading alginate biofilm. It is of both fundamental and applied interest to understand the function of these enzymes. Many of the alginate related enzymes have been characterized, but the putative family 7 alginate lyase PA1784 is hitherto not described in the literature. The goal of the present project is to determine the properties of PA1784 and investigate its putative activity towards alginate. Activity of the purified enzyme has been investigated towards alginate from macroalgae and a mucoid *P. aeruginosa* variant using a variety of qualitative and quantitative techniques. The work is in progress and the most recent results will be thoroughly presented and discussed.

FA6 - Characterization of a β-1,3-glucanase from *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic human pathogen that causes acute and chronic lung infections in individuals with compromised immune systems. It is highly persistent in clinical settings due to its ability to produce complex biofilms that contributes to protection from antibiotics. An important constituent of some *P. aeruginosa* biofilm variants is an exopolysaccharide called PsI. This polysaccharide consists of glucose, mannose and rhamnose moieties coupled by β-1,3, and is regularly branched with mannose attached by α-1,2 bonds to the main chain mannose.

The main objective of this thesis is to characterize a putatively secreted family 50 glycoside hydrolase *P. aeruginosa* enzyme with an endo-1,3-β-glucanase activity and its possible activity towards Psl.

The gene encoding endo-1,3-β-glucanase, called *Pa*GH50A was cloned into a pNIC-CH vector, containing a hexa-histidine tag for convenient purification, that was subsequently transformed into *E.coli* strain BL21 for expression. Protein expression was successful and approximately 4,1 mg pure protein per L culture was obtained.

FA7 - Screening for modulators of vesicular monoamine transporter 2 activity in transfected Hek293 cells using a fluorescent substrate.

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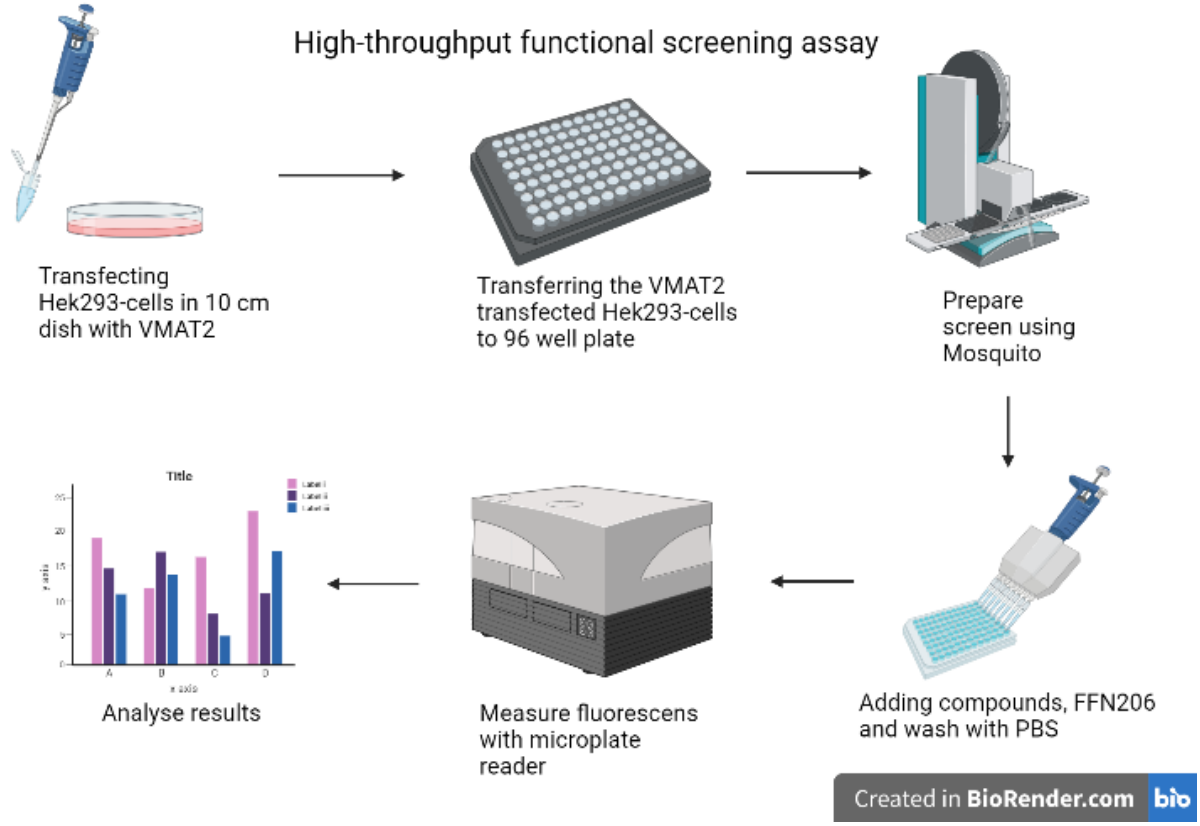
The monoamine neurotransmitters, such as dopamine (DA), serotonin, histamine, and epinephrine are accumulated in synaptic vesicles, before being released into the synapse upon neurotransmission. The membrane transporter vesicular monoamine transporter 2 (VMAT2) is responsible for this packaging. VMAT2 is a secondary active antiporter that use the vesicular proton gradient generated by V-type ATPase to transport monoamine up its concentration gradient. VMAT2 can thus act as a regulator of monoamine homeostasis and is a potential drug target for neuronal diseases, such as hyperkinetic movement disorders and Parkinson's disease. We aim to identify new modulators of VMAT2 by high-throughput functional screening. This screening is based on measuring uptake of a fluorescent VMAT2 substrate in Hek293 cells transiently expressing VMAT2. Promising candidates from this screening will be further validated using substrate uptake assays, and the direct interaction between VMAT2 and hit compounds will be studied using biophysical approaches.



FA8 - The role of protein-protein interactions in neurodegenerative disease

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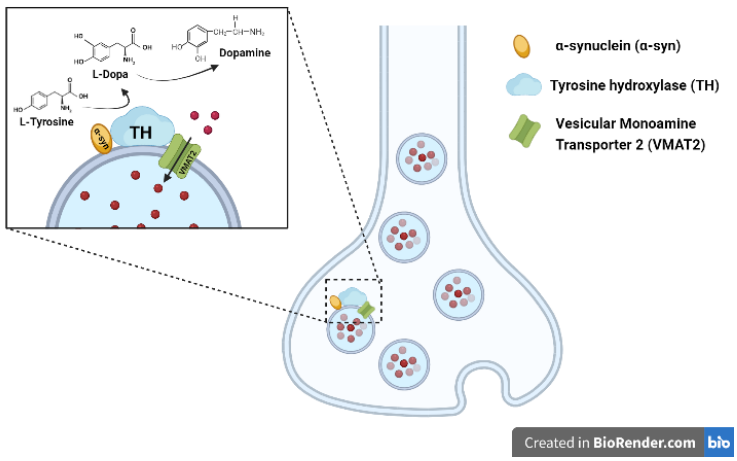
Parkinson's disease (PD) is a neurodegenerative disease that affects the brain's ability to control movements and is often accompanied by cognitive impairment. PD is a characterised by a progressive death of dopamine (DA)-producing neurons in the substantia nigra, decline in Tyrosine Hydroxylase (TH), formation of α -synuclein (α-syn) containing Lewy bodies and a loss of dopamine signalling in the striatum.

TH is the enzyme catalyzing the conversion of tyrosine to L-DOPA, the rate limiting step in the DA synthesis. L-DOPA is then further converted to DA by aromatic L-amino acid decarboxylase (AADC). These enzymes have been shown to form a functional protein-protein interaction (PPI) complex at the vesicular membrane with α-syn, and the vesicular monoamine transporter 2 (VMAT2).

We hypothesize that this complex is coordination synthesis and storage of DA into synaptic vesicles, two processes that are vital for DA signaling. Further, recent results suggest that they are also transported together along axonal microtubules from the cell soma to presynaptic terminals. However, knowledge of how these proteins are forming a complex, and the mechanism behind this complex formation is still lacking.

The goal of this project is to contribute to understand the mechanisms behind the protein-protein interaction between TH and α-syn, and their association with vesicular membranes. I will also be studying the effect of α-syn on the DA feedback inhibition of TH. To achieve this, I will be performing biophysical and structural studies on recombinantly expressed and purified TH and α-syn proteins, including methods such as Bio-Layer interferometry (BLI), enzymatic assays, size exclusion chromatography – multi angle light scattering (SEC-MALS) and crosslinking experiments.



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FA9 - FISH&CRISPR: Discovery and characterization of CRISPR-associated endonucleases from low-temperature organisms

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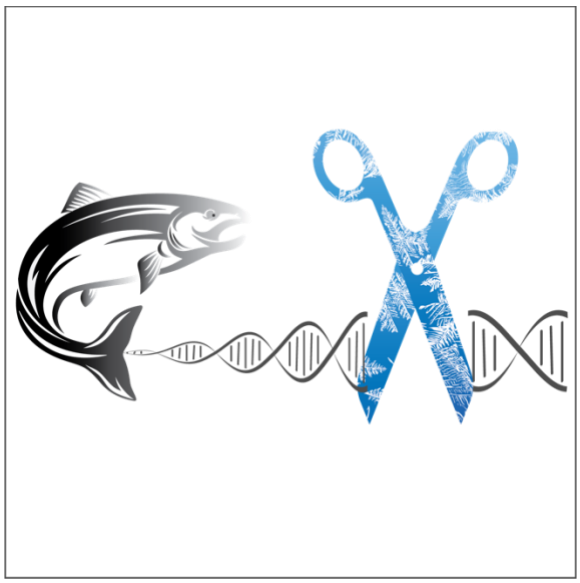
The CRISPR-Cas genome editing system has revolutionized molecular biology, providing an array of biotechnological tools for carrying out precision genome modification and regulation in eukaryotic cells. One limitation of the system at present is that available tools are developed from and optimized for mesophilic organisms, which limits their utility in extremophilic organisms. Although thermophilic Cas9 homologs have been developed and verified for use at high temperatures, less attention has been given to the low-temperature end of the spectrum. This paucity of available psychrophilic genome editing tools is particularly problematic for researchers of cold-blooded eukaryotes such as fish biologists, as both the current enzymes and fish cells lose viability at elevated temperatures. This project is one of three interdisciplinary components of the UiT Strategic-Funded 'FISH&CRISPR Innovative strategies to improve salmon health' which aims to establish a platform for the development of a low-temperature CRISPR-Cas genome editing system optimized for salmonids. In this project, the goal is to discover and develop one or more CRISPR-associated endonucleases for effective and precise genome editing at low temperatures. Candidates were selected by bioinformatics analysis of genomes of psychrophilic bacteria. Further, expression and purification of the enzymes are under optimization. These enzymes will be characterized including biophysical properties, enzymatic properties and structure. The ultimate goal is verification that the Cas/sgRNA complexes are able to perform efficient and accurate editing in salmon cell lines at low temperatures, and optimization of genome editing protocols.



FA10 - Gut microbiota and antibiotic resistome changes in the first year of life of preterm infants supplemented by probiotics while at NICUs

Ahmed Bargheet, Eirin Esaiassen, Erik Hjerde, Jorunn Pauline Cavanagh, Tanja Pedersen, Jannicke H. Andresen, Siren I. Rettedal, Ragnhild Støen, Britt Nakstad, Claus Klingenberg, Veronika K Pettersen

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Annually, roughly 15 million infants are born prematurely (before 37 weeks of pregnancy) across the world, representing a global preterm birth rate of nearly 11%. Antibiotics use contributes significantly to the expansion of the gut antibiotic resistance genes collection (resistome) in addition to increasing the risk of infections by collateral killing or inhibiting the commensal bacteria (gut microbiota) and increasing opportunistic bacterial pathogens. Since preterm infants almost universally receive early antibiotic therapy, it is crucial to understand how the intervention influences gut microbiota development. In Norwegian neonatal intensive care units, clinically tested probiotics have become a standard of care for reducing gastrointestinal inflammation and infections in extremely preterm newborns. However, little is known about their impact on gut microbiota assembly and whether they might mitigate the expansion of resistome. In this study, we analyzed fecal samples collected from 76 infants; 31 antibiotics probiotics-supplemented extremely preterm (APEP) infants born at <28 weeks gestation, 35 antibiotics non-probiotics-supplemented very preterm (ANPVP) infants (28–31 weeks gestation), and 10 healthy full-term control (FTC) infants. Metagenomic sequencing data were analyzed to determine taxonomic and resistome composition at four different time points; Day 7, Day 28, Day 120, and Day 365. Antibiotic administration decreased the richness and evenness of the gut bacterial community. Moreover, antibiotics use delayed the transition to a mature microbiome configuration, as compared to samples from healthy term infants, which displayed higher community richness and interconnectivity. On the other hand, the administration of the probiotics significantly decreased the abundance of antibiotic resistance genes (ARGs) carried by the gut bacteria at Day7. ARGs were significantly correlated with microbiota composition indicating that the microbial phylogeny shapes the infant gut resistome. These result suggest that probiotic supplementation in early life can mitigate resistome expansion driven by antibiotic therapy.

FA11 – Investigating the mechanisms of colonization resistance in the early life human gut microbiome: A metabolomic approach

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Antimicrobial resistance (AMR) poses a great threat worldwide. Antibiotics is one of the therapeutics most widely used on children due to their undeveloped immune systems. However, it is estimated that 50% of the antibiotic use is unnecessary. Antibiotics perturb the microbiome in the gut which may lead to severe infections by AMR pathogens. Although there is agreement in that a healthy microbiome avoid severe infections in a higher degree than an antibiotic treated one, little is known about the molecular mechanisms that cause this. Throughout this PhD, multi-omics tools will be used to investigate and determine genetic and metabolic markers that influence colonization of AMR pathogens. Stool samples from two infant cohort studies will be characterized by omics approaches. First, the effect of probiotic therapy on preventing infection by resistant *Enterobacterales* in Tanzanian children will be investigated. Both the microbiome and the metabolome will be studied. Secondly, the fecal metabolome of infants receiving antibiotics will be determined before and after therapy and associated with DNA sequencing data. Biochemical interactions between gut bacteria and AMR pathogens will also be investigated. For the above-mentioned studies to be possible, optimization of mass spectrometric analysis utilizing untargeted LC-MS systems will be performed. The goal of this thesis is to characterize biological markers that can be used as targets for preventing bacterial AMR colonization thus avoid overuse of traditional antibiotics.

FA12 - The early gut and skin microbiomes of Atlantic salmon yolk sac fry originating from two different microbial source communities

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The fish microbiome plays important roles in protecting the host, providing nutrition, and enabling proper development. However, very little is known about the early colonization in fish and more knowledge is needed here to promote positive host-microbe interactions. We therefore characterized the development of the skin and gut microbiomes of Atlantic salmon fry throughout the yolk sac stage, examined the influence of the source microbiota, and investigated the potential for manipulating the early microbiome. Our yolk sac fry was reared under two microbial conditions: conventionally raised (CVR; non-sterile conditions) or conventionalized (CVZ; re-colonized germ-free fry). By 16S rRNA amplicon sequencing, we characterized the gut and skin microbiomes at 6, 9, and 13 weeks post hatching (wph) in a first experiment, followed by a second experiment where 16S rDNA amplicon sequencing was used to investigate manipulations of the microbiota at 8 wph by addition of the bacterial pathogen *Yersinia ruckeri* or a bacterial commensal, *Janthinobacterium* sp.

In both experiments the gut and skin microbiomes were highly dissimilar between the CVR and CVZ group on ASV level, however, both groups were dominated by Proteobacteria and Bacteroidetes. The inter-individual variation was high, and a strong flask effect was observed especially for the CVZ samples. The microbiomes changed significantly over time and the richness increased. The gut and skin microbiomes did not differ in the first experiment (no significant differences in the alpha- and beta-diversity), but strong differences were observed in the second experiment. The addition of bacterial strains had minor effects on the microbial communities, indicating resilience of the microbiome already in early developmental stages. In conclusion, we showed that the source microbial community plays an important role in the initial colonisation of Atlantic salmon yolk sac fry, indicating a potential for managing the early microbiomes of the fry.

FA13 - Depletion of immobilized crude oil in temperate Norwegian waters

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Oil exploration and production in marine areas increase the risk of accidental oil spills. Degradation of oil by natural or stimulated methods is an important part of oil spill risk assessment, but field data on oil degradation processes like photo-oxidation and biodegradation are limited. This project aims to fill essential knowledge and data gaps on field-based degradation of oil in temperate marine regions and to point out variations in the degradation rate connected to the accompanying microbial community composition and light regime. In this project two different field systems have been tested to determine the influence of various carrier materials and the influence of light on oil degradation. The field systems were deployed in a harbour area in Trondheim between December and March (2019/2020) with regular samplings. Hydrophobic adsorbents and clay beads were used as carriers for the oil, respectively. The oil was prepared as thin films on the adsorbents and used to coat the out- and inside of clay beads. Lab studies have shown that biodegradation of oil compounds in these immobilized films may relate to degradation of oil dispersions. Chemical analysis of the oil has shown that certain compounds are more likely to be degraded under the presence of light, while others display no reaction to light exposure. Furthermore, it was observed that the degradation pattern of certain oil compounds of the covered adsorbents is more similar to the clay beads samples (only covered) than to the light exposed adsorbents, which leads to the conclusion that the presence or absence of light has a greater effect than the type of carrier material used. Microbial influence on the degradation has been characterized by Illumina sequencing of 16S rDNA amplicons to examine potential correlations between the microbial community dynamics and degradation patterns of oil components.

FA14 - Mining of enzyme targets for clinical control of vancomycin-resistant *Enterococcus faecium*

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A rise in antibiotic resistance among bacteria is making many concerned that we are entering into a post-antibiotic era with untreatable bacterial infections and the consequences this will have on human health. Since the 70s the enterococci have become significant causes of drug resistant hospital acquired infections, especially by the species *E. faecalis* and *E. faecium.* In the last two decades infection by *E. faecium* has been on the rise. Especially prevalent in *E. faecium* is the resistance against vancomycin which is a last resort antibiotic used to treat serious infection by bacteria resistant to most other drugs. Infections by vancomycin-resistant enterococci (VRE) has increased dramatically in the latest years.

There is therefore a need for development of new treatment options for VRE that can increase the susceptibility to antibiotics or anti-virulence agents and take into consideration the adaptability and versatility of *E. faecium* as an opportunistic pathogen. We hope that by looking into the proteome of *E. faecium* and comparing enzyme activities in clinical and commensal strains we will be able to identify enzyme candidates that have the potential to be chemotherapeutic targets that can allow for specific targeting of pathogenic *E. faecium* strains.

To investigate the proteome of *E. faecium* we apply the chemoproteomic technique called activity-based protein profiling (ABPP) which applies chemical probes to bind active enzymes expressed by the bacteria. Using these probes, we can get insight into the active proteome and explore protein expression between different strains and under different conditions. We are identifying and exploring serine hydrolases, glycosidases, ATPases/kinases, and penicillin binding proteins that could lead to future treatment options for *E. faecium* infections.

Keywords: activity-based probes, *Enterococcus faecium,* serine hydrolases, glycosidases, ATPases, kinases, penicillin binding proteins

FA15 - Identification Of Serine Hydrolase Virulence Factors In Methicillin-resistant - *Staphylococcus aureus* By Using Carmofur-derived Activity-Based Probe

Md Jalal Uddin and Christian Lentz *

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Staphylococcus aureus is a major human pathogen and a leading cause of bacterial infections worldwide. Many patients suffer from *S. aureus* infections that are often chronic and are never fully cured by antibiotics due to the ability of *S. aureus* to persist in biofilms or other protected niches. Therefore, developing novel diagnostic strategies and treatment options for life-threatening *S. aureus* infections is an urgent priority. Chemical probes, so-called activity-based protein profiling (ABPP) are a powerful technique for deciphering the specific functional enzymes in bacterial systems. Activity-based probes (ABPs) are functionalized enzyme inhibitors that can rapidly and irreversibly bind with their target enzymes by covalently modifying the active site of catalytically active enzymes via specific chemical reaction. This study aimed to identify novel (functional) enzymatic activities in *S. aureus* during biofilm formation.

Competitive ABPP was used to identify serine hydrolases/functional enzymes and inhibitors activity in live *S. aureus* by treating intact cells with carmofur/5-fluorouracil and fluorescent carmofur-derived ABP (G11) followed by analysis of labeled proteins by SDS–PAGE analysis. LC-MS/MS was used to identify target enzymes using biotin-tagged G11 & transposon mutant strains for target validation. MIC & time-kill assay of carmofur and 5-fluorouracil was performed by the broth microdilution method.

The carmofur-derived ABP (G11) showed broad activity in targeting functional enzymes on biofilm-promoting growth conditions. The G11 labeled the secreted serine hydrolase/lipase and other fluorophosphate-binding serine hydrolases (fphB, fphE, and fphF). Most bands labeled by the G11 can be competed out by pretreatment with the unlabelled parent inhibitors carmofur and active drug, 5-fluorouracil and both drugs showed the same antibacterial activity (MIC-5µM) & time-dependent killing against *S. aureus* USA 300.

The G11 could be used for targeting & inhibiting a group of hydrolytic enzymes whose functions are likely to be important for various aspects of cellular physiology and host-pathogen interactions.

Keywords: activity-based probes, serine hydrolases, *Staphylococcus aureus*

FA16 - Identification of TCS kinases involved in host-microbe interactions

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The regulation of most bacterial stress responses, as orchestrated by tightly coordinated and balanced dynamics between kinases and phosphatases. The bacterial Two-Component Systems (TCSs) are among the response architectures that mediate the response to environmental stress factors and the expression of effective adaptive mechanisms. In a basic configuration, TCSs are composed of a membrane-bound sensor kinase which autophosphorylates in response to external factors. Transfer of the phosphoryl group to the response regulator allows the modulation of gene expression. Kinases in bacterial TCS remain promising therapeutic targets due to their conserved active sites and cell surface localization. Staphylococcus species possess more than 20 TCS with global regulon and regulatory cross-talk, making the identification and characterization of specific kinases roles complicated.

To identify and characterize specific kinases involved in bacterial colonization and infection of human cells, we use Activity-Based Probes (ABPs), composed of chemically engineered modules for targeting the conserved active site of enzymes. ABPs contain tags that enable identification, enrichment, purification, and visualization of the labeled targets. ABPs combined with Mass Spectrometry allow for high throughput qualitative and quantitative validation of low abundant proteins and posttranslationally modified enzymes, whose functional profiles do not correlate with their gene expressions.

Aside from identifying specific kinases involved in host-microbe interaction, we have successfully used Kinases-targeted ABPs to map out the proteome network of essential kinases regulating key metabolic and virulence determinants in the bacteria under various stress conditions.

FA17 - Activity-based protein profiling of *Staphylococcus aureus* exposed to blood

Clement Ajayi[1], Md Jalal Uddin[1], Christian Lentz[1], Mona Johannessen[1]

[1]Research group for Host-microbe Interactions, Department of Medical Biology, Faculty of Health Science, UiT – The Arctic University of Norway, Tromsø, Norway.

Staphylococcus aureus is an important human pathogen. It has many virulence factors that facilitate invasion of host cells, evasion of host immune responses and tissue damage. These virulence factors are expressed during bacterial adaptation to the dynamic conditions in the host. An adequate understanding of these virulence factors is required to better understand *S. aureus* pathogenesis.

Activity-based protein profiling (ABPP) is a robust technique enabling the identification of active proteins during bacterial interaction with the host. The underlying principle of ABPP is the use of specially designed small molecules called activity-based probes (ABPs) to identify specific protein target(s) within a complex proteome. ABPs consist of an electrophilic reactive group linked with a reporter tag.

Here, a rhodamine-tagged serine hydrolase probe was used to identify putative virulence protein during bacterial exposure to blood. *S. aureus* strains grown on blood agar plates were harvested and lysed via mechanical disruption. Cell lysates were incubated with the ABP. The probe-labeled proteome was resolved on SDS polyacrylamide gel and visualized by in-gel fluorescence using the Typhoon fluorescent image scanner. In-depth identification of active proteins was achieved by labeling bacterial cell lysates with a biotin-tagged serine hydrolase probe. Enrichment of probe labeled target was performed. The enriched samples were subjected to trypsin digestion and the resulting peptides were analyzed by LC-MS analysis.

From the proteomic data analysis, a series of putative *S. aureus* proteins which are active upon bacterial exposure to blood were identified. Functional assays to further confirm the contribution of these proteins to *S. aureus* virulence during host interaction are in the pipeline.

Taken together, our results have identified novel targets that might be important for *S. aureus* virulence in the blood. Increased understanding of these targets will help in the development of alternative therapeutic interventions to combat *S. aureus* infections.

FA18 - PX Oslo - UiO Structural Biology Core Facilities: Who are we and how can we help you?

Nikolina Sekulić & Ute Krengel

University of Oslo

The UiO Structural Biology core facilities are run by PX-Oslo, located at four locations in Oslo (Department of Chemistry, Department of Biosciences, NCMM and the Institute of Clinical Medicine/OUS). These facilities comprise the Oslo node of the national Norwegian Macromolecular Crystallography Consortium NORCRYST. We provide service and advice regarding structural biology techniques. We can assist in the whole process from construct design to structure determination. Our core competence is in protein crystallography, therefore we call us **PX-Oslo.** We have regular access to synchrotrons and other cutting-edge international facilities that allow us to collect high quality data.

In addition to ***protein crystallography***, we also work with ***X-ray and neutron scattering (SAXS/SANS)***, ***quantitative biophysical characterization, structural mass spectrometry (HDX-MS) and cryo-EM***. Although NMR spectroscopy is not our core competence, we tightly collaborate with NMR experts and are happy to provide advice and contacts.

Your first consultation will be free of charge, so don't be shy to contact us!

We also offer a 5-pt course in Structural Biology Techniques (KJM5320/9320), sponsored by BioCat, for Master's and PhD students.

Flash Talk Session B

FB1 - The application of LPMOs during enzymatic saccharification of woody biomass

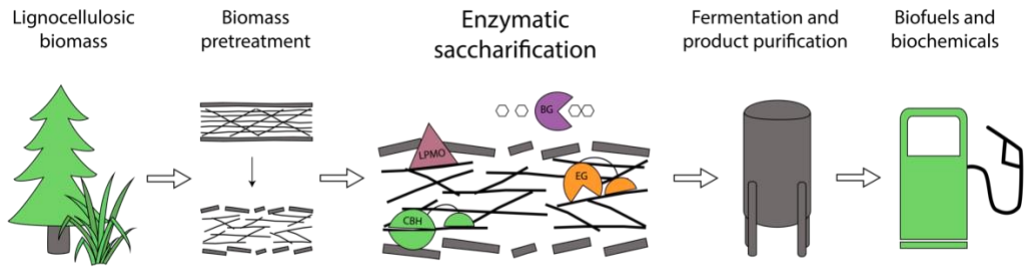
Camilla Fløien Angeltveit, Eirik Kommedal, Vincent G. H. Eijsink, Svein Jarle Horn

Faculty of Chemistry, Biotechnology, and Food Science, Norwegian University of Life Sciences (NMBU), Ås, Norway

Today, the environmental impacts of human activities have far-reaching implications, especially in the form of climate change. Hence, the importance of developing long-term low-carbon valorization pathways utilizing materials such as lignocellulosic biomass is growing. The production of biofuels and biochemicals from lignocellulosic material is demanding because of the robust biomass structure. Enzymatic saccharification of this recalcitrant material into platform sugars, which allows subsequent fermentation into desired products, is regarded as one of the main bottlenecks in lignocellulosic biorefineries.

Enzymatic saccharification has usually been conducted by enzyme cocktails consisting of different endo- and exo-acting cellulases, but over the last decade, novel enzymes called lytic polysaccharide monooxygenases (LPMOs) have been included in these enzyme blends (Chylenski et al. 2019). LPMOs are of particular interest because they act on the surface of the polysaccharides and cut directly into the crystalline structure, improving the accessibility of the recalcitrant material for classical hydrolytic enzymes (i.e., cellulases). However, since LPMOs are redox enzymes that need electrons and an oxygen co-substrate (O_2 or H_2O_2), it is challenging to optimize the activity of these crucial enzymes, for example, because they are prone to auto-catalytic inactivation when supplied with too much co-substrate (Bissaro et al. 2017). The presence of redox-active lignin in biomass complicates matters because lignin can both activate LPMOs by donating electrons to the enzyme and generate the H_2O_2 co-substrate.

We are optimizing lignocellulose saccharification processes, with a particular focus on the impact of pretreatment technologies and lignin content on LPMO activity. One goal is to better understand how to control the *in situ* production of H_2O_2 to maintain efficient and stable saccharification of polysaccharides over time. Considering that lignin is light-sensitive, we also address the impact of light exposure on process efficiency. These are all important factors for accomplishing sustainable valorization of lignocellulose.



Bissaro, B., Røhr, Å. K., Müller, G., Chylenski, P., Skaugen, M., Forsberg, Z., Horn, S. J., Vaaje-Kolstad, G. & Eijsink, V. G. (2017). Oxidative cleavage of polysaccharides by monocopper enzymes depends on H 2 O 2. *Nature chemical biology*, 13 (10): 1123.

Chylenski, P., Bissaro, B., Sørlie, M., Røhr, Å. K., Várnai, A., Horn, S. J. & Eijsink, V. G. (2019). Lytic polysaccharide monooxygenases in enzymatic processing of lignocellulosic biomass. *ACS Catalysis*, 9 (6): 4970-4991.

FB2 - Python coding for high-throughput analysis of stopped-flow spectroscopic data that shed light on the redox chemistry of lytic polysaccharide monooxygenases

Ole Golten[1], Ivan Ayuso-Fernandez[1], Kelsi Hall[1], Morten Sørlie[1], Åsmund Røhr Kjendseth[1], Vincent G.H. Eijsink[1]

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Lytic polysaccharide monooxygenases (LPMOs) are mono-copper enzymes capable of degrading recalcitrant carbohydrates such as cellulose, chitin, xylan and starch and they have sparked a large interest amongst researchers due to their fascinating catalytic properties and relevance to biomass conversion [1]. Since their discovery in 2010 [2] intense research on LPMOs has been performed, but the reaction mechanism remains enigmatic. Moreover, recent studies showing that H_2O_2 is the preferred co-substrate, rather than O_2 [3, 4], has created controversy and debate in the field and revealed a need for more in-depth characterisation of LPMOs using, for example, stopped-flow (i.e., real time) spectroscopic techniques. In the case of LPMOs, fluorimetry and stopped-flow rapid mixing may be used to assess the redox state of the copper atom coordinated in the catalytically conserved histidine T-brace[5]. The considerable amount of raw data created in such ventures is daunting and would benefit from automatized data analysis.

Herein we show a computational workflow using the Python coding language that vastly increases data analysis from raw output files for a more streamlined characterization of LPMOs. To reproducibly analyse large sets of LPMO kinetic data, we wrote a modular Python script which can perform experimental curve fitting to user defined functions with a time complexity measured in linear time $O(n)$. The Python analysis of stopped-flow experiments decreased the analysis time from 30 minutes to 20 seconds for each determined rate constant. In addition, the use of Python to analyse stopped-flow data files removes potential user bias and increases reproducibility.

Using our stopped-flow set-up, which is part of the Norwegian Macromolecular Crystallography Consortium (NORCRYST), and the newly developed automated data management pipeline, we have carried out kinetic analyses of the redox properties of multiple LPMOs and examples will be presented.

1. Chylenski, P., Bissaro, B., Sørlie, M., Røhr, Å. K., Várnai, A., Horn, S. J. & Eijsink, V. G. H. (2019) Lytic Polysaccharide Monooxygenases in Enzymatic Processing of Lignocellulosic Biomass, *ACS Catalysis.* **9**, 4970-4991.
2. Vaaje-Kolstad, G., Westereng, B., Horn, S. J., Liu, Z., Zhai, H., Sørlie, M. & Eijsink, V. G. H. (2010) An Oxidative Enzyme Boosting the Enzymatic Conversion of Recalcitrant Polysaccharides, *Science.* **330**, 219-222.
3. Bissaro, B., Røhr, Å. K., Müller, G., Chylenski, P., Skaugen, M., Forsberg, Z., Horn, S. J., Vaaje-Kolstad, G. & Eijsink, V. G. H. (2017) Oxidative cleavage of polysaccharides by monocopper enzymes depends on H2O2, *Nature Chemical Biology.* **13**, 1123-1128.
4. Rieder, L., Petrović, D., Väljamäe, P., Eijsink, V. G. H. & Sørlie, M. (2021) Kinetic Characterization of a Putatively Chitin-Active LPMO Reveals a Preference for Soluble Substrates and Absence of Monooxygenase Activity, *ACS Catal.* **11**, 11685-11695.
5. Bissaro, B., Streit, B., Isaksen, I., Eijsink, V. G. H., Beckham, G. T., DuBois, J. L. & Røhr, Å. K. (2020) Molecular mechanism of the chitinolytic peroxygenase reaction, *Proceedings of the National Academy of Sciences.* **117**, 1504.

FB3 - Hole hopping through tryptophan chains in LPMOs

Trond S. Moe*, Ivan Ayuso-Fernandez*, Åsmund K. Røhr*, Morten Sørli*, Kelsi Hall*, Ole Golten*, Vincent G.H. Eijsink*

*Norwegian University of Life Sciences

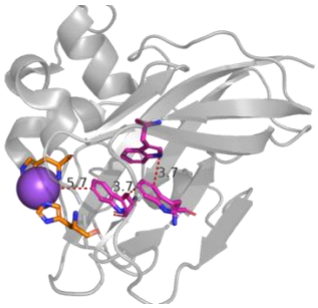
07.01.2022, Norwegian University of Life Sciences

Lytic polysaccharide monooxygenases' (LPMOs) ability to break down non-soluble polysaccharides is vital in nature and of great interest in biomass processing. In the decade since their discovery (Vaaje-Kolstad et al. 2010) significant effort has been put into understanding the mechanisms that enable these enzymes to oxidatively cleave glycosidic bonds.

Wide scope bioinformatic analysis of known crystal structures in the protein data bank (PDB) has shown that chains of spatially connected tyrosine and tryptophan residues occur commonly in natural proteins (Gray and Winkler 2015). Among such proteins, redox enzymes are overrepresented, indicating a possible role of aromatic amino acid chains in protecting such enzymes from oxidative damage. Moreover, the participation of such chains in hole hopping pathways has been shown experimentally for some of these enzymes.

Examination of the *Serratia marcescens* chitin-active AA10 LPMO (also known as CBP21) revealed that three tryptophan residues (Trp81, Trp91 and Trp151) form a chain through the core of the protein, from the buried side of the catalytic copper to the surface. Given that Trp81 and Trp151 are conserved among AA10 LPMOs, we generated enzyme variants to study the role of these amino acids. The tryptophane residues were mutated to tyrosine and methionine, with different redox properties, and functional characterization of the mutant enzymes is in progress.

We use standard activity and binding assays, as well as analyses of redox properties using stopped-flow kinetics, to determine mutational effects on LPMO properties. By modeling electron flow through the amino acid chains using the program EHPath (Teo et al. 2019) we hope to link the observed changes in biochemical properties with changes in the modeled electron flow. Our results will be useful to understand the mechanism of action of these industrially relevant enzymes, and to aid in the design of LPMOs that are less prone to oxidative damage.



FB4 - Biorefining of Enzymatically and Microbially Tailored Side Streams from Plant Protein Production

Philipp Garbers[1], Catrin Tyl[1], Svein J. Horn[1], Bjørge Westereng[1], Svein H. Knutsen[2]

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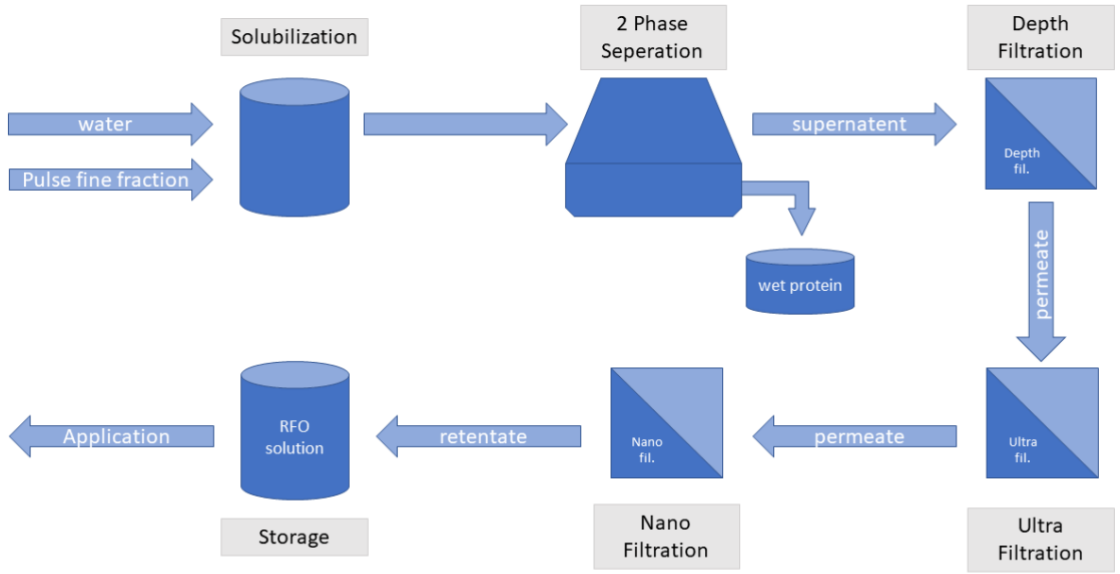
[2] Nofima AS, Ås, Norway

To reach sustainability and health goals, there is an increased interest in plant-based food products such as meat-analogues based on plant protein. During the production of plant protein, for example from legumes like beans, pea or soy, huge side streams arise that need to be valorised. These side streams are mainly the starch fraction and fiber rich hulls, but the protein fraction also contains other constituents that could be utilized. These are depending on the type of processing and feedstock, different types and amounts of anti-nutrients like FODMAP's, vicine & convicine or phytic acid for example. These need to be treated during further processing for food production in order to avoid problems for consumers and increase acceptability and sustainability. The main FODMAP constituents in legumes are the raffinose family oligosaccharides (RFOs), thus being the initial focus.

To access the RFOs, they first need to be isolated from the fractionized plant materials by biorefining. During the biorefining it is important to keep the protein structure intact, so it is still useable for food production while at the same time producing a highly concentrated fraction of RFOs. The suggested biorefining pathway is shown in the attached picture. After successful biorefining further research on the utilization of the extracted RFOs can be done. To reach the overall aim of providing green technologies, the focus of the utilizations will be on biotechnological processes like enzymatic treatment or fermentations with microorganisms to produce valuable products for food production or beyond.



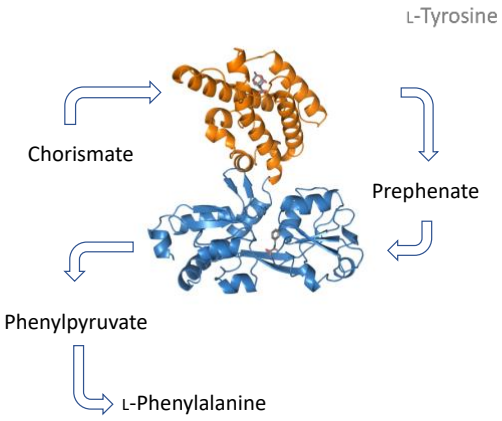
FB5 - Structure-function analysis of bifunctional metabolic enzymes from the shikimate pathway

Tamjidmaa Khatanbaatar[1], Christian Stocker[2], Gabriele Cordara[1], Peter Kast[2], Ute Krengel[1]

[1] *Department of Chemistry, University of Oslo, Oslo, Norway*

[2] *Laboratory of Organic Chemistry, ETH Zurich, Zurich, Switzerland*

Chorismate mutase (CM) is a key enzyme of the shikimate pathway, responsible for the biosynthesis of the aromatic amino acids L-Tyr and L-Phe in bacteria, fungi, archaea, and plants. It catalyzes the Claisen rearrangement of chorismate to prephenate. In some organisms, CMs are active on their own; in others, CM is coupled to other enzymes of the pathway, either covalently or non-covalently. Here, we present the investigation of the CM-fusion enzyme with cyclohexadienyl dehydratase (CDT), one of the subsequent enzymes in the pathway, which converts prephenate to phenylpyruvate and thereby channels it towards the biosynthesis of phenylalanine. Specifically, I show the structure-function relationship of the CDT-CM fusion enzyme from *Janthinobacterium sp. HH101,* investigated by X-ray crystallography, small-angle X-ray scattering, molecular dynamics, and enzyme kinetics.



The chorismate mutase (CM) and cyclohexadienyl dehydratase (CDT) are two sequential enzymes in the shikimate pathway. While they are usually found as separate proteins, CDT-CM fusion enzymes are found in several organisms.

FB6- Characterizing antimicrobial bacteriosin activity in *Staphylococcus haemolyticus*

Runa Wolden[1], Kirill Ovchinnikov[2], Hermoine J. Venter[1], Dzung B. Diep[2], Jorunn Pauline Cavanagh[1]

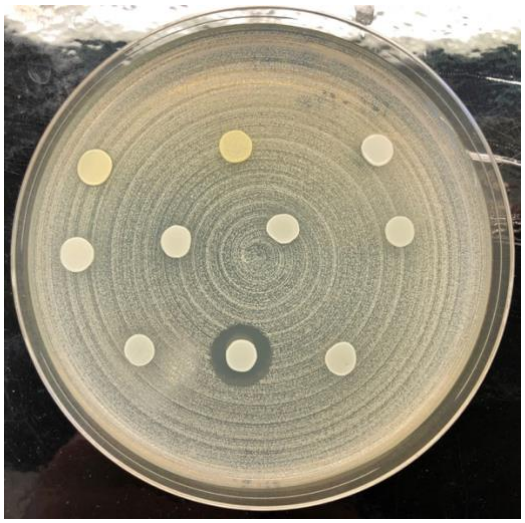
[1] Paediatric Research group, Department of Clinical Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway
[2] Laboratory of Microbial Gene Technology, Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), Ås, Norway

Antimicrobial resistance (AMR) is a global health threat, and the clinical pipeline of new antimicrobials is dry. Bacteriocins are ribosomally synthesized peptides produced by bacteria to inhibit growth of closely related bacterial species. As bacteriocins are possible novel antimicrobial agents, we investigated putative antimicrobial bacteriocin activity of *Staphylococcus haemolyticus* isolates. *S. haemolyticus* is a skin commensal that has gained increased attention as an emerging pathogen of hospital infections, and it is often multidrug-resistant.

We screened overnight cultures from clinical and commensal isolates of *S. haemolyticus* for bacteriocin activity against three indicator strains: *Lactococcus lactis* (control), clinical *S. haemolyticus*, and clinical *Staphylococcus aureus*. Cell-free supernatants were prepared from the test isolates that displayed antimicrobial activity against any of the indicator strains. We chose one isolate for further experiments due to its promising activity. pH, protease sensitivity and heat-stability were investigated. Cationic exchange eluate was applied to a reverse-phase chromatography column connected to a purifier system. The activity of the purified fractions was tested against ESKAPE pathogens and a broad range of Gram-positive indicators.

Three of 174 *S. haemolyticus* overnight cultures inhibited the growth of indicator strains. One of the isolates showed antimicrobial activity against all three indicator strains when using supernatant. The activity was pH stable, heat stable (121 °C) and proteinase sensitive (trypsin), which are typical characteristics of a bacteriocin. The purified fractions displayed activity against a broad range of Gram-positive indicators, including *S. aureus*, *S. haemolyticus*, *Enterococcus faecalis* and *Listeria monocytogenes*. There was no activity against *Gram-negative bacteria.*

We have found a putative new antimicrobial bacteriocin produced by a commensal *S. haemolyticus* isolate. Planned future work include sequencing the bacteriocin operon, heterologous expression, mode of action and biofilm experiments. New antimicrobial compounds are important to fight the AMR problem.



FB7 –The role of capsule variants in Staphylococcus haemolyticus on immune response and biofilm formation

M.O.K. Christensen[1], H.J. Venter[1], H.N. Granslo [1, 2], C. Klingenberg[1, 2] & J.P. Cavanagh[1]

[1]Pediatric Infection Group, Department of Clinical Medicine, faculty of Health Sciences, UiT - The Arctic University of Norway, Tromsø Norway

[2]Dept. of Pediatrics, University Hospital of North Norway and University of Tromsø

Staphylococcus haemolyticus is one of the most common causes of sepsis in premature babies and children with cancer. *S. haemolyticus* is part of our normal microbiome and has only a few virulence factors. However, clinical *S. haemolyticus* isolates are highly antibiotic-resistant and often attach to surfaces and form a biofilm layer on invasive catheters and medical implants. Little is known about the strategies *S. haemolyticus* uses to establish an infection or evade the host immune system. In order to understand what distinguishes invasive from commensal S. *haemolyticus* isolates, our research group has previously performed a comparative genome and pangenome analysis. During this analysis, three novel capsule polysaccharide operons were identified. It has been shown that polysaccharide capsules protect *S. haemolyticus* against the host immune defence. Until now, this has only been demonstrated for one type of capsule. Here we present our plan for this project.

In this project, we will investigate whether the different capsule types are associated with different biofilm-producing abilities and phenotypic capsules, and whether they differ in the level of protection from phagocytosis. This will be examined by a combination of molecular manipulation (such as knockouts and heterologous expression), microscopy, biofilm assays, phagocytosis assays and by the use of various in vitro and ex vivo models. Depending on the findings, animal models may also be included.

We hope these results and knowledge will help identify new therapeutic targets and better treatment strategies for sepsis with *Staphylococcus haemolyticus* in premature babies and children with cancer.

FB8

Characterization of methyltransferase-like21C (METTL21C) in muscle

Kamilla Nygård, Erna Davydova, Mads Bengtsen and Pål Ø. Falnes
Department of Biosciences, University of Oslo, Norway

The addition of methyl groups to biomolecules is one of the most frequent chemical modifications in cells and ~200 known and putative methyltransferases are encoded by the human genome. Proteins are frequent targets of methylations, and the modification plays an important role in regulating and optimizing protein function. In skeletal muscle tissue, several non-histone proteins are known to be methylated. However, for most of these, the biological relevance is unknown, and the responsible methyltransferase remains elusive. Methyltransferase-like21C (METTL21C) is a lysine methyltransferase implicated in muscle biology, with a few reported substrates. However, a clear *in vitro* methyltransferase activity in muscle cells remains to be demonstrated. Here, we set out to study the function of METTL21C in muscle cells.

To investigate the ability of METTL21C to methylate proteins in muscle extracts, we performed *in vitro* methylation assays where the methylation reactions were performed in the presence of [^{3}H] S-adenosylmethionine (AdoMet), and protein methylation detected with fluorography. We found that METTL21C methylated a single protein with a molecular weight different from previously reported substrates. Notably, the extent of methylation was found to be considerably higher in METTL21C knock-out extracts than in wild-type extracts, indicating that this represents a *bona fide* substrate of METTL21C.

Next, we will try to uncover the identity of this novel METTL21C substrate, using mass spectrometry-based techniques. Once the substrate has been identified, we aim to characterize the *in vitro* activity of METTL21C on this substrate, including the actual methylation site.

FB9 - Functional studies in *C. elegans* to elucidate the role of METTL13 in mRNA translation during health and disease.

Melanie L. Engelfriet[1], Jędrzej M. Małecki[1], Anna F. Forsberg[1], Pål Ø. Falnes[1] and Rafal Ciosk[1,2].
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[2] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland

The novel methyltransferase-like protein 13 (METTL13) is a dual methyltransferase that has been shown to methylate the N-terminus and Lys55 of the eukaryotic elongation factor 1 alpha (eEF1A) in human cells. Previous studies have shown that depletion of METTL13 seems to be dispensable in healthy tissues with its absence causing no effect on protein synthesis or proliferation. In contrast, depletion in several human cancer cell lines reduces tumorigenesis by inhibiting global protein synthesis and cell proliferation. Here, we use *C. elegans* as a model organism to assess if the function of METTL13 and its mechanism in health and disease is evolutionarily conserved. Using methyltransferase assays and mass spectrometry, we show that METTL13 is responsible for methylating the same eEF1A targets in the *C. elegans*. Using a loss-of-function mutant worm, we show that loss of METTL13 causes no impact when it comes to development, fertility, life span, or global protein synthesis. Interestingly, the only time a differential phenotype was observed between wild-type and METTL13 mutant worms, was in the context of cancer. Due to the germline being the only tissue in *C. elegans* that is capable of tumorigenesis, we induced tumor formation in the germline of wild-type and METTL13 mutant worms. Here we noted that the tumorous germline looked less severe in the absence of METTL13. Together, our findings confirm that the biochemical function and the mechanism of METTL13 in healthy and cancerous tissue are conserved from human to nematode.

FB10 - Exploring the transcriptional regulation of *C. elegans* hibernation

Frida Forsberg[1], Agnieszka Chabowska-Kita[2], Yanwu Guo[1] and Rafal Ciosk[1,2]

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2. Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

C. elegans can survive sever cold by entering a hibernation-like state and multiple studies have been performed in order to identify key factors in *C. elegans* hibernation. However, little is known about the factors in charge of the underlying gene regulation of *C. elegans* hibernation. We have previously identified two transcription factors DAF-16/FOXO and PQM-1 that promote cold survival in *C. elegans* by upregulating FTN-1, which enhance cold survival by detoxifying ROS-generating iron species. To examine if these transcription factors are the drivers of the global changes in gene expression seen upon cold exposure we created *daf-16*, *pqm-1*and *daf-16; pqm-1* double mutants. Through RT-qPCR analysis of key up- and down regulated genes in the mutant strains, we found that there were no significant changes in gene expression patterns upon cold exposure as compared to the wild type. This suggests that although DAF-16/FOXO and PQM-1 play a role in enhancing cold survival they are not in charge of the gene regulation of *C.elegans* cold response. In addition, we designed worm strains expressing GFP under the *lips-11* promotor. Lips-11 is a lipase, which is significantly upregulated upon cold exposure. This reporter strain will allow us to examine all potential transcription factors involved in the gene regulation of *C. elegans* cold response. One hypothesis that we are currently testing with said reporter strain is based on the assumption that a putative repressor inhibits *lips-11*gene expression at 20 °C. Upon cold exposure, this repressor is inhibited allowing for *lips-11* transcription. To test this we have stated an EMS mutagenesis screen at 20°C were after mutagenesis any mutants inappropriately expressing GFP-taged LIPS-11 could be due to inactivation of a *lips-11* repressor. This will allow us to examine if gene expression changes seen during *C. elegans* hibernation is due to the inactivation of a repressor.

FB11 - Phage display to improve the lives of celiac disease patients

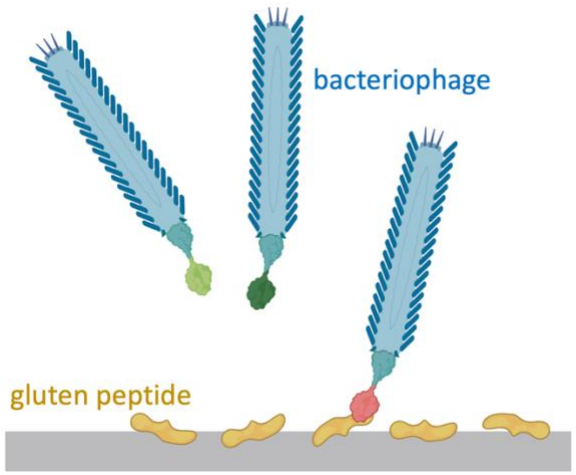
Julie E. Heggelund[1,2], Lene S. Høydahl[1,2], Ludvig M. Sollid[1,2]

[1] Department of Immunology, University of Oslo and Oslo University Hospital, Norway

[2] KG Jebsen Coeliac Disease Research Centre, University of Oslo, Norway

Celiac disease is an inflammatory disease of the small intestine caused by hypersensitivity to cereal gluten proteins. Gluten proteins are resistant to gastrointestinal digestion, and the immune response is preferentially directed against certain epitopes. Plasma cells specific for such gluten epitopes can be isolated from gut biopsies of celiac disease patients. The only effective treatment of the disease is a lifelong strict gluten-free diet. Effective monitoring of gluten in foodstuff is important – for celiac disease patients to be able to stay gluten free and for the food industry to provide safe food. The current state-of-the-art method is using the R5 competitive enzyme-linked immunosorbent assay (ELISA). This method can also be used to detect gluten in stool and concentrated urine samples, to monitor whether patients have been inadvertently exposed to gluten. However, this test does not fully represent gluten immunogenicity in celiac patients.

By using the method of phage display, we aim to make new antibodies with improved affinity and retained specificity, for detection of gluten in food and patient samples. We have generated celiac patient derived monoclonal antibodies from gut plasma cells that bind gluten peptides. These monoclonal antibodies were used as starting materials. By diversifying the complementarity-determining regions, we have created libraries of potential binders. The strongest binders will be selected using bacteriophage M13 and specific gluten peptides. We have finished the first selection rounds and will test the resulting antibodies against binding to gluten peptide using ELISA.



FB12 - Gamma radiation induces locus specific changes to chromatin accessibility in zebrafish embryos

Leif Christopher Lindeman[12] , Selma Hurem[12], Erik Rasmussen[12], Dag Brede[12], Jarle Ballangby[12], John Arne Dahl[3], Jan Ludvig Lyche[12], Peter Aleström[12],

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Gamma radiation is able to induce damage to nucleic acids and proteins. Chromatin, consisting of both DNA and DNA associated proteins, is the functional form of DNA, and a potential target for epigenetic effects. Here we investigate the potential of gamma radiation to induce changes to chromatin during embryogenesis. To do so, zebrafish embryos were exposed to 1, 10 and 40 mGy/h gamma radiation (Cobalt-60 from 2.5 hour post fertilization (hpf) to 5.5 hpf. At 5.5 hpf the transcriptome (RNA-seq) and the endonuclease Tn5 accessible open chromatin was analyzed (ATAC-seq). In addition, Chromatin immunoprecipitation (ChIP) of H3K4me3, H3K9me3, H3K9ac and H3K27me3 were carried out at the 5.5 hpf stage and compared to unexposed embryos. ATAC-seq revealed a dose response relationship between enriched chromatin and radiation dose rate, wherein the amount of Tn5 accessible chromatin at the transcription start site (TSS) decreased with increasing radiation dose rate, suggesting a global inhibition of gene expression. In the gene body (GB), no global difference in accessible chromatin is observed. ChIP analysis on single gene levels, revealed a dose response on H3K4me3, H3K9ac, H3K9me3 and H3K27me3 to gamma radiation. Although many genes were compacted at TSS, some genes also obtained a more open chromatin signature. This suggests major TSS rearrangements of chromatin after exposure and reveals insight into molecular stress-responses in reaction to gamma radiation.

FB13 - Inosine independent roles of Endonuclease V

Solbakk, Hannah Winther[1]; Augestad, Ingrid Lovise[1]; Berges, Natalia[1]; Lærdahl, Jon Kristen[1,2]; Lien, Guro Flor[1]; Nawaz, Meh Sameen[1]; Alseth, Ingrun[1]

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Adenosine in RNA is deaminated by specific enzymes in the cells to yield inosine. This changes the coding properties of the RNA and is important for transcriptome diversity. Human endonuclease V (ENDOV) is a ribonuclease specific for inosines that is found in most species. Despite this evolutionary conservation and a well-defined catalytic activity, the *in vivo* role of ENDOV is poorly understood. To learn more, we performed a crosslinking immunoprecipitation (iCLIP) assay with ENDOV and among abundant targets was yRNA4. yRNAs are short non-coding cytoplasmic RNAs that to our knowledge, do not contain inosines. yRNA makes together with Ro60, ribonucleoprotein particles important for RNA surveillance and is tightly linked to autoimmune disease. Here we present data supporting the interaction between ENDOV and yRNA4 by the use of synthetic and endogenous RNA substrates as well as protein partner- and microscopic analyses. We suggest that the role of ENDOV is to regulate the level of small non-coding RNAs to fine-tune gene expression under stress.

FB 14 - Antimicrobial and antibiofilm potential of marine bacteria

Ataur Rahman, Andrea Iselin Elvheim, Christoffer Sivertsen, Ida Kristine Østnes Hansen, Bjarne Landfald, Hans-Matti Blencke, Tor Haug, Klara Stensvåg

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Sessile marine invertebrates like sponges, sea anemones, and tunicates serve as surfaces for biofouling, and many of these organisms have developed strategies to battle against fouling and maintain a clean exterior surface. Marine invertebrates also host a range of microbial symbionts that often produce compounds protecting their host. This project aims to establish novel basic knowledge of antibiofilm compounds from rich marine sources, specifically marine bacteria in Arctic and sub-Arctic regions. We have isolated and cultured 158 strains of marine bacteria from a research cruise north of Svalbard. Identification was performed by 16S rRNA sequencing followed by MALDI-TOF. The antimicrobial potential of marine bacteria was screened by co-culture against human pathogen relative reference bacteria. Antimicrobial compounds were extracted from the potential marine bacteria, and the antimicrobial activity of isolated compounds was also screened against human pathogen relative reference bacteria. The structure of those compounds will be elucidated by LC-MS followed by NMR. Furthermore, the mechanism of action as antimicrobial will be explored using different *in-vitro* and *in-vivo* methods along with in-house developed biosensor strains. Antibiofilm activity profiling will be performed using known biofilm-producing strains to screen isolated compounds from marine bacteria. Additionally, functional analysis and interpretation of transcriptomic data from RNA-seq will be used to determine the mechanism of the antibiofilm activity of novel compounds. Innovative solutions for biofilm inhibition/eradication will be achieved through isolated compounds. This will ultimately contribute to the improvement of public health and the prevention of marine biofouling.

FB15 - Characterizing Enzymes in The Ectoine Biosynthesis Pathway

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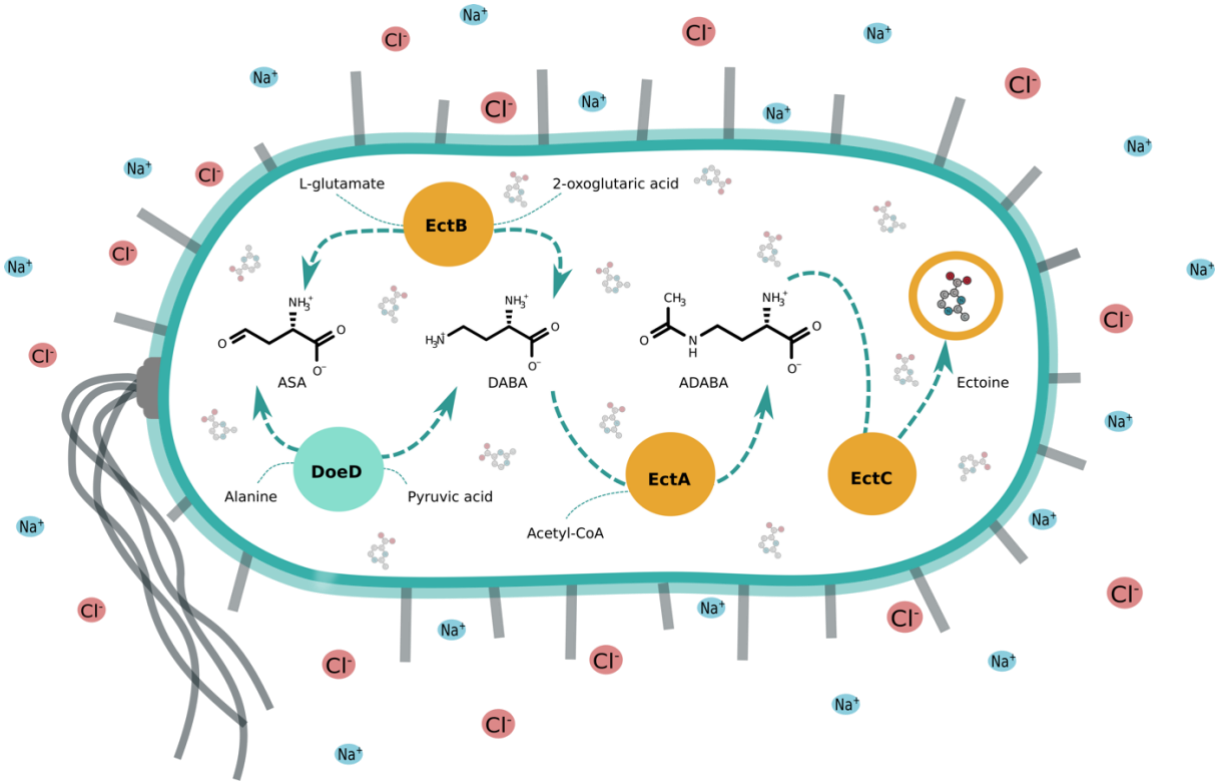
[1]The Norwegian Structural Biology Centre (NorStruct) at the Department of Chemistry, UiT The Arctic University of Norway.

We aim to characterize enzymes associated with the biosynthesis of a small organic compound called ectoine, by doing structural and functional studies. Ectoine belongs to a group of molecules known as osmolytes, which are small organic compounds that are actively accumulated in, amongst others, bacterial cells to protect them against environmental stress. Ectoine has many possible biotechnological applications, for example, it can protect cells against changes in temperature and osmolarity but also against drying and freezing, it has high water-retaining abilities, and is shown to have a stabilizing effect on proteins.



FB16 - Genetic Mapping by Whole-genome sequencing

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Forward genetics in model organisms remains a powerful tool to reveal genes involved in a specific biological process. Whole-genome sequencing and bioinformatic analysis facilitate the mapping for responsible mutation. The goal of genetic mapping is to locate phenotype-causing SNPs (Single nucleotide polymorphisms) in the genome. The linkage between SNPs and mutated gene is distance-dependent. SNPs that are not linked to the phenotype-causing SNP/gene are diluted after backcrossing and phenotype selection. Only SNPs that are close to the phenotype-causing SNP/gene are enriched, which is revealed by SNP analysis. In case of viable mutants with recessive mutations, the mutants were homozygous for the phenotype-causing SNPs, so all corresponding sequencing reads displayed those SNPs. However, in case of the recessive sterile mutant, the progeny of heterozygous mutant were collected. Based on Mendelian genetics, these were ¼ wild-type, ½ heterozygous, and ¼ sterile. On average, half of the corresponding sequencing reads displayed the phenotype-causing SNP and the other half the wild-type sequence. In what we called “the heterozygous SNP frequency-based mapping”, or Het-Map for short, the location of phenotype-causing mutation was pinpointed based on the increased co-occurrence between the phenotype and “heterozygous” SNPs.

Poster abstracts

P1 - Revisiting the chitinase of *Pseudomonas aeruginosa*

Per Kristian Thorén Edvardsen, Fatemeh Askarian and Gustav Vaaje-Kolstad

Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences

A study by Folders et. al in 2001 showed that *Pseudomonas aeruginosa* encode and possibly secretes a chitinase (a protein that can degrade chitin). Several strains of *P. aeruginosa* encode this protein, and bioinformatic analysis of this putative enzyme showed similarity to chitinases from other organisms, including the well-characterized chitinase ChiC1 from *Serratia marcescens*, which has been characterized earlier in the host laboratory. Intriguingly, *P. aeruginosa* is not able to grow on chitin as the sole carbon source. This begs the question, what is the role of this enzyme in *P. aeruginosa*? The aim of this project is to fully characterize and elucidate the biological function of this protein by investigating its chitinolytic potential in comparison to chitinases with purely catabolic roles. We will also analyze substrate promiscuity by glycan array screening and activity screens. Initial enzymatic data will be presented and discussed.

P2 - Do you know what FEBS is and has to offer you?

Winnie Eskild

Dept of Biosciences, University of Oslo, Norway

FEBS stands for “The Federation of European Biochemical Societies”. As member of NBS you are also member of FEBS, one of Europe’s largest scientific organizations in the molecular life sciences. It has close to 40,000 members across 39 member states each of which has its national biochemical society. As NBS member you have access to FEBS fellowships of various duration and travel grants in connection with FEBS supported scientific meetings, advanced scientific courses, workshops on higher education issues. FEBS also provides support and travel grants for participants at the annual FEBS Congress and young scientist forum. FEBS is a self-financed charity based on four peer reviewed scientific publication with somewhat differing profiles: the FEBS Journal, FEBS Letters, Molecular Oncology and FEBS Open Bio. A new online forum for the molecular life science community is available comprising channels, rooms, profiles and more for free use by you.

P3-

IBA – National Graduate School in Infection Biology and Antimicrobials

Director: Mike Koomey
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NBS meeting participant: Runa Wolden

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Partner institutions:
University of Oslo (UiO)
Norwegian University of Science and Technology (NTNU)
Norwegian University of Life Sciences (NMBU)
University of Tromsø: The Arctic University of Norway (UiT)
Norwegian Institute of Public Health (NIPH / FHI)
University of Bergen (UiB)

The National Graduate School in Infection Biology and Antimicrobials (IBA) is a research school for PhD students and postdocs registered at Norwegian universities. We hold courses in infectious diseases and infection biology and arrange annual meetings. Our network connects the research environments of infectious diseases and infection biology throughout Norway.

Our program co-fund expenses related to participation in courses and research stays in Norway and abroad. IBA is funded by the Research Council of Norway and is coordinated from the University of Oslo.

We offer several courses, such as: Principles of infection biology, Vaccinology, Antibiotics and Antibiotic Resistance, Pathogenic Immune Evasion Strategies, One Health Approach to Zoonotic Infectious Diseases, Advanced antimicrobial resistance, Creating Scientific Illustrations, Scientific Writing and Career Webinars.

All PhD students in IBA are also members of the IBA-PhD association, where the goal is to ensure the academic and social interests of the students enrolled in IBA. The association organizes workshops and social activities and nominate student representatives to the IBA board.

P4 - ATRAID, a highly glycosylated protein localized to membranous cellular compartments

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ATRAID (All-trans retinoic acid-induced differentiation factor) is a well conserved, poorly characterized gene, its expression induced by all-trans retinoic acid. Currently, two transcripts are listed in NCBI, the largest coding being NM_001170795.4 (transcript 3, isoform C) and the shorter NM_016085.5 (transcript 1, isoform A). At the protein level the most striking difference between them is a postulated N-terminal signal peptide in isoform C lacking from isoform A. Both isoforms are predicted to contain several N-glycosylation sites. ATRAID is shown to be involved in cell cycle arrest by inhibiting the expression of CCND1 (cyclin D1), and thereby suppress proliferation. Also, it binds to NELL-1 at the nuclear envelope of human osteoblasts, affecting osteoblast proliferation and differentiation. Furthermore, in human dental pulp cells, this ATRAID-NELL1 complex inhibits proliferation by downregulating cyclin D1. In ARPE-19 cells ATRAID promotes cellular senescence, and the expression of ATRAID is increased in RPE-cells derived from aged mice. ATRAID locates to the mitochondria in ARPE-19 cells and binds to NRF2. Overexpression induces morphological changes in mitochondria, disrupting its function and increasing apoptosis. Moreover, it is shown that ATRAID locates to the lysosome where it binds to the lysosomal proteins LAMP1 and SLC37A3. Complex of ATRAID/SLC37A3 are required for releasing of nitrogen-containing-bisphosphates (N-BPs) from the lysosome to the cytoplasm.

In this study, we aim to further characterize this protein, its location and function. To reach this purpose we produced transgenic cell lines expressing ATRAID with a c-terminal Flag-tag. Western blotting revealed ATRAID to be highly N- glycosylated. The combination of subcellular fractionation and ICC showed that ATRAID is absent from the cytosol and the nucleus and is located mainly in the plasma membrane and in intracellular vesicles or compartments. Further studies will elucidate more of its biological functions.

P5 - Revealing the Changing Lipidome of Cellular Senescence

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Lipid molecules, which possess a wide range of functions in fundamental cellular processes such as transcription, apoptotic induction, and protein distribution, are increasingly emerging as critical players in cellular senescence and healthy ageing. The significant diversity of lipids found in the cell is accomplished through the coordinated action of enzymes that ultimately lead to the incorporation of various aliphatic chains to different polar headgroups, collectively known as the lipidome. However, despite increasing research interest, lipidomic studies have lagged behind proteomic and transcriptomic studies, leaving significant gaps in our understanding of the lipid players in the senescent phenotype, such as the activation of crucial senescence pathways (e.g. p16, p53), energy metabolism, the composition of the senescence-secretory phenotype (SASP), and the remodelling of the cell membrane.

By integrating transcriptomic data with two complementary lipidomic methods, we are able to assess how the lipid landscape changes with age, how this contributes to the senescence phenotype, and the impact this may have on cellular activity in the context of age-related pathology.

P6 - Marine bivalves and their symbionts as a novel source of cold-adapted wood-degrading enzymes

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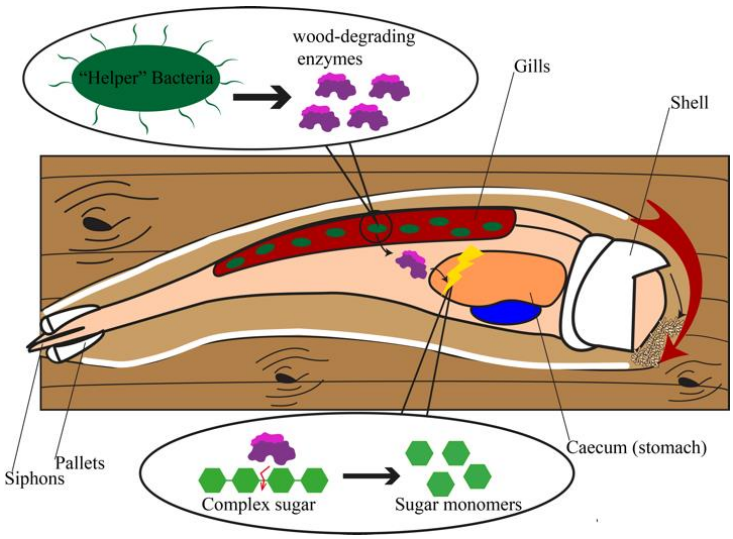
The leftover biomass from forestry and agricultural activities has the potential to be converted into highly valuable products. However, the rigid structure of this biomass makes it very resistant to degradation. Therefore, enzymes with unique and improved properties are needed to efficiently degrade and convert this biomass.

Marine bivalves like shipworms and Xylophaga are experts at degrading driftwood at sea. Bacteria living in their gills aid them in this process by secreting wood-degrading enzymes. In our research group, we aim to understand more about the nature and characteristics of wood-degrading enzymes from marine, cold-adapted organisms. How do they adapt their enzymes to their environment, and how do they differ from land-based wood-degraders?

Cold-adapted marine organisms have been significantly less studied than their land-based counterparts. Learning more about the mechanisms behind specific properties, like high salt tolerance and efficient degradation even at reduced temperatures, could be invaluable to the continued development of the green industry.

This work has mainly been focused on enzymes from a bacterial metagenome from the gills of a *Psiloteredo megotara* – a common shipworm along the Norwegian coast. Starting from this metagenome, we have used various bioinformatics tools to design and construct ten target enzymes, of which six have been successfully expressed in *E. coli*. One of these enzymes, a GH5 family cellulase, has been further characterized and shows significant retained activity at temperatures as low as 7 °C, making it a good candidate for use in delicate industrial processes where reduced temperatures and better degradation control is needed.



Career Session Talk

For a Life after the PhD: My three months internship experience at Grünenthal in México.

Lisa Schroer, UiO

More than 70% of all PhD students do not want an academic position following their graduation (NIFU Survey of PhD students in Norway, 2017*). Unfortunately, the education during our three to four year scientific education leaves little space to exploring the sectors outside academia. Therefore, I took it into my own hands to conduct an internship at a pharmaceutical company to get to know not only how a company is structured, but also how and where my analytical and organizational skills that I have acquired during my PhD fit into the industrial sector. Follow my presentation if you want to learn about my short, but intense experiences at Grünenthal in Mexico City!

* I. Reymert, K. Nesje and T. Thune; A survey of doctoral candidates in Norway, English summary; NIFU; Proj. Nr. 12820736; http://hdl.handle.net/11250/2445865 (complete Norwegian report); 2017