BioCat Annual Conference

ABSTRACT BOOKLET

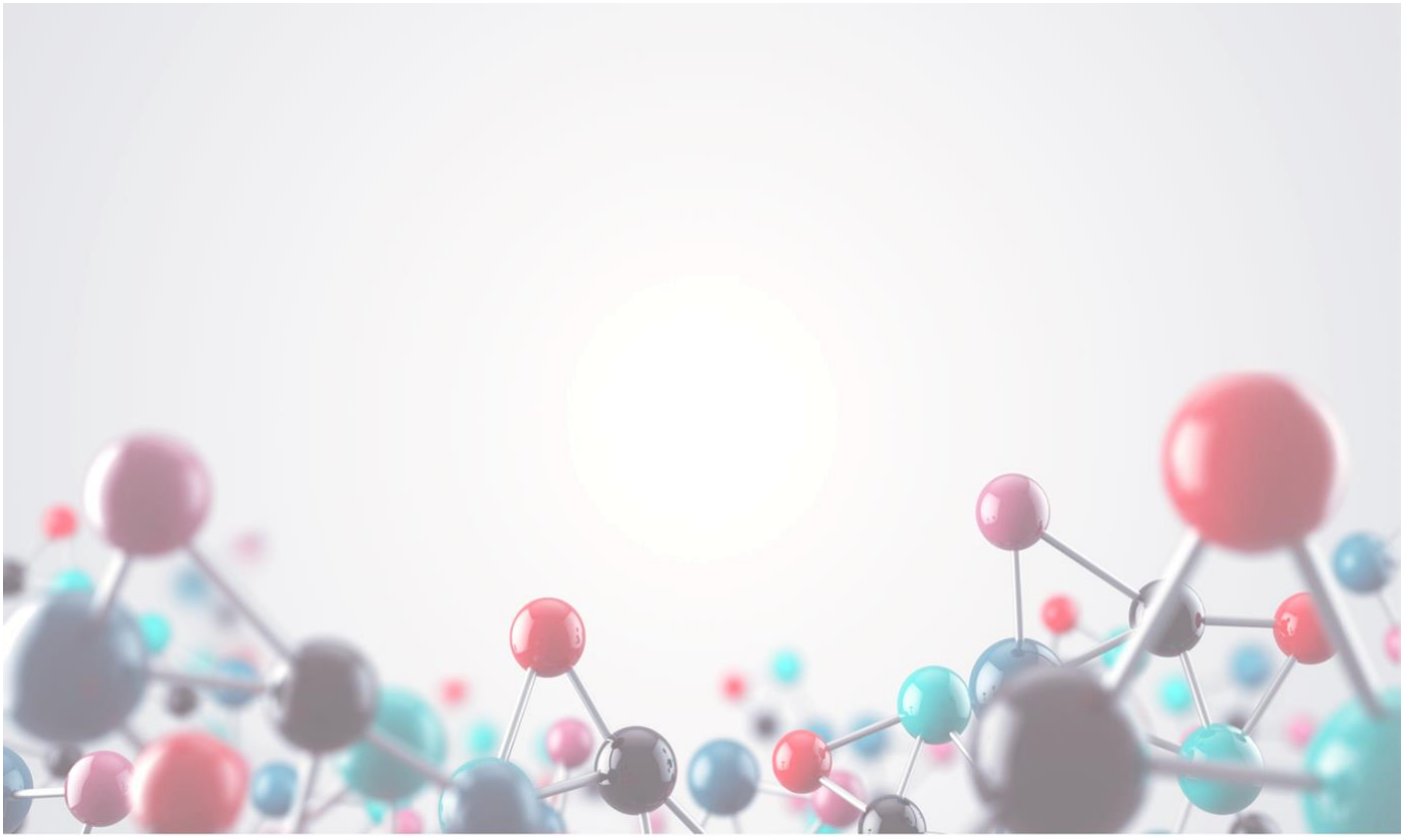
Stavanger, 11th-13th September 2024

BioCat
Norwegian Graduate School in Biocatalysis

BioCat Annual Conference

BioCat
Norwegian Graduate School in Biocatalysis

ORAL PRESENTATION ABSTRACTS



WEDNESDAY – SESSION 1 (kl. 14.45)

3-MINUTE FLASH PRESENTATIONS

10-MINUTE STANDARD PRESENTATIONS

Computational study of Xanthan Gum Glycosyltransferase GumK

Davide Luciano[1], Jochen Schmid[2] & Gaston Courtade[1]

[1] Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, Trondheim, Norway, [2] Institute of Molecular Microbiology and Biotechnology, University of Münster, Münster, Germany

Exopolysaccharides are a diverse class of molecules with a wide range of industrial applications. Xanthan gum, a heteropolysaccharide synthesized by the Gram-negative bacterium *Xanthomonas campestris*,[1] is a significant contributor to this class of polymers. Its versatile mechanical properties make it useful in various fields such as the food, material, and pharmaceutical industries.[2] Modifications of the polysaccharide are being studied to modulate its properties for more specific applications.[3] In this study, we present a computational approach to identify key residues in the active site of GumK, a glycosyltransferase involved in xanthan biosynthesis, that influence the dynamics and activity of the enzyme. We describe the dynamics of the two domains and the residues involved in the binding of the natural substrates. Our computational protocol is applicable to other glycosyltransferases within the pathway and can elucidate enzyme selectivity. This insight can inform bioengineering strategies to produce new variants of xanthan gum.

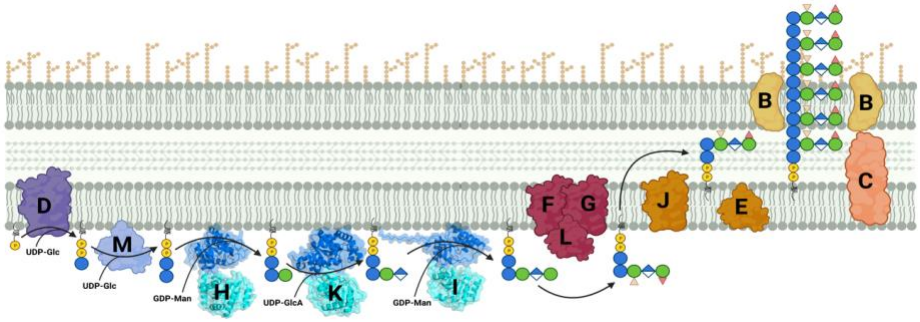


Figure 1. Set of proteins involved in the biosynthesis of xanthan gum. The target enzymes of this work are Gum H, K, I, which are involved in the assembly of the side chains of the gum.

Keywords: Glycosyltransferase, GTB, Xanthan Gum, biased MD

References:

1. Anke Becker and Federico Katzen and Alfred Pühler and Luis Ielpi (1998) Xanthan gum biosynthesis and application: a biochemical/genetic perspective. *Applied Microbiology and Biotechnology*, 50: 145-152.
2. Chaturvedi Surabhi, Kulshrestha Sanchita, Bhardwaj Khushboo and Jangir Rekha (2021) A Review on Properties and Applications of Xanthan Gum. *Microbial Polymers: Applications and Ecological Perspectives*: 87-1072.
3. Patel, Jwala and Maji, Biswajit and Moorthy, N. S. Hari Narayana and Maiti, Sabyasachi (2020) Xanthan gum derivatives: review of synthesis, properties and diverse applications. *RSC Adv,* 10(45): 27103-27136.

Structural modeling of antibody variant epitope specificity with complementary experimental and computational techniques

Eva Smorodina[1], Oliver Crook[2], Rahmad Akbar[1], Puneet Rawat[1], Dario Segura Pena[3], Nikolina Sekulic[3], Ole Magnus Fløgstad[3], Khang Lê Quý[1], Brij Bhushan Mehta[1], Johannes Loeffler[4], Monica Fernandez-Quintero[4], Hannah Turner[4], Andrew B. Ward[4] & Victor Greiff[1]

[1]Department of Immunology, University of Oslo and Oslo University Hospital, Oslo, Norway, [2]Department of Statistics, University of Oxford, Oxford, [3]Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, Faculty of Medicine, University of Oslo, Oslo, Norway, [4]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, USA

Antibodies are key therapeutics but the principles behind diverse paratopes binding to the same epitope remain unexplained. An insufficient understanding of the structural rules behind antibody-antigen binding, due to a lack of experimentally resolved structures, leads to the current inability to characterize antibody variants binding in silico. Here we propose a rule-based antibody design that relies on a thorough understanding of epitope-paratope interactions, in contrast to generative design based on millions of trials and errors. We identified the epitope of five affinity-verified Trastuzumab variants using cryo-EM and position-resolved HDX-MS. Rigid models alone are insufficient for accurate antibody-antigen modeling while molecular dynamics simulations with computational analysis of the complex conformations succeed in replicating and complimenting experimental findings. Structural parameters calculated based on geometry, surface, and biochemical properties were able to distinguish between high and low binders. We highlight the possibilities of AI in antibody and antibody-antigen structure modeling, demonstrating the limitations of various language-based models to predict and understand antibody variants. Overall, our study explains the binding mechanisms of the variant sequences, showing how antibodies with diverse sequences share similar antigen-binding rules.

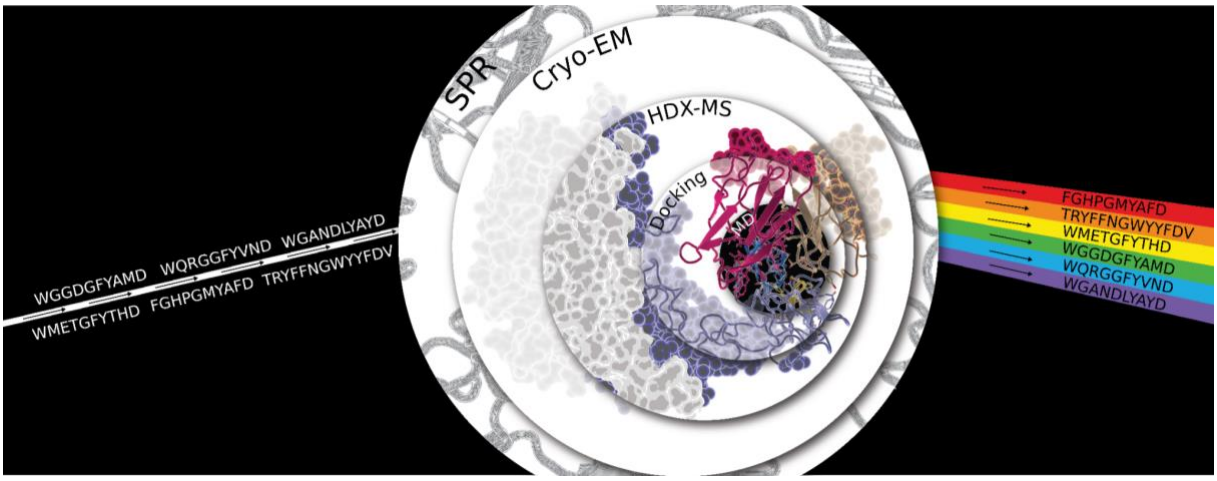


Figure 1. Different levels of complexity surrounding structural rules behind antibody-antigen binding. We start from a more general understanding of the interaction kinetics with SPR, then identify the global binding site with cryo-EM, refine the region with HDX-MS to achieve peptide-level resolution, and move forward towards residue- and atom-wise resolution with computational techniques like docking and molecular dynamics.

Valorization of protein-rich poultry side-streams: spectroscopy-based monitoring of enzymatic hydrolysis for collagen content determination

Greta Nardini[1], Kristian Hovde Liland[2], Sileshi Gizachew Wubshet[3], Nils Kristian Afseth[3] & Kenneth Aase Kristoffersen[1]

[1]NMBU – Norwegian University of Life Science, KBM Faculty, P.O. Box 5003, NO-1432, Ås, Norway, [2]NMBU – Norwegian University of Life Science, REALTEK Faculty, P.O. Box 5003, NO-1433, Ås, Norway, [3]Nofima – Norwegian Institute of Food, Fisheries and Aquaculture Research, P.O. Box 210, NO-1431, Ås, Norway

Enzymatic Protein Hydrolysis (EPH) is a well-established and versatile technology for the valorization of protein from food industry side-streams. Low-value cuts from poultry, a protein-rich biomass, are transformed into high-value protein hydrolysates with different physicochemical properties. One example is collagen-enriched hydrolysates used in food products, pharmaceuticals, and cosmetics.[1]

Process monitoring and feed-forward control mechanisms are crucial to optimize yield and product quality from EPH processes.[2] The amino acid composition is an important process parameter, and along with the molecular weight distribution (MWD), it is a key parameter in determining the hydrolysates' physicochemical properties. In poultry side-stream valorization, collagen solubilization is another key parameter and can be followed using Hydroxyproline, an amino acid almost exclusive to collagen.[3]

The gold standard for amino acid composition analysis is the chromatographic method.[4] Recently, however, there has been a growing interest in spectroscopic techniques which are green, rapid, and non-destructive, making them ideal for real-time measurements.[2,5,6] Fourier Transform Infrared spectroscopy (FTIR), a vibrational spectroscopy technique, has successfully been applied to monitor industrial processes to assess relevant analytical parameters like MWD and collagen content.[7,8] While vibrational spectroscopy is not suitable for predicting the amino acid composition, Nuclear Magnetic Resonance (NMR) spectroscopy is. NMR offers deeper insights because it is superior to FTIR in probing metabolites and low molecular components, it also provides more reliable quantitative (qNMR) data.[9,10]

In this study, the performance of FTIR and NMR spectroscopy has been compared with classical methods for investigating collagen content in EPH samples from poultry side-streams.

References:

1. K. A. Kristoffersen et al., Food Chem 2022, 382, DOI 10.1016/j.foodchem.2022.132201.
2. S. G. Wubshet et al., Food Bioproc Tech 2018, 11, 2032–2043.
3. R. E. Neuman et al., Journal of Biological Chemistry 1950, 184, 299–306.
4. European Commission Regulation, No 152/2009 of 27 January 2009.
5. S. Shin et al., Food Chem 2021, 352, DOI 10.1016/j.foodchem.2021.129329.
6. M. S. Eissa et al., TrAC 2024, 170, DOI 10.1016/j.trac.2023.117435.
7. I. Måge et al., LWT 2021, 152, DOI 10.1016/j.lwt.2021.112339.

Mode of Action of AlgE1 – A Modular Mannuronate C-5 Epimerase

Agnes B. Petersen[1], Anita Solem[1], Gerd Inger Sætrom[1], Håvard Sletta[2], Finn L. Aachmann[1] & Anne Tøndervik[2]

[1]Department of Biotechnology and Food Science, Norwegian University of Science and Technology. Trondheim, Norway, [2]Department of Biotechnology and Nanomedicine, SINTEF Industry. Trondheim, Norway

Alginate epimerases convert β-D-mannuronate (M) to its C-5 epimer α-L-guluronate (G) in alginates. Alginates are linear anionic polysaccharides produced by brown seaweed and some bacteria. In alginates M and G are organized in blocks of M (polyM), blocks of G (polyG), and in alternating blocks of MG (polyMG). [1]

Alginate epimerases are modular, Ca^{2+}-dependent enzymes that work in a processive manner [1] The alginate epimerase AlgE1 from *Azotobacter vinelandii* consists of two catalytically active A-modules (A_1 and A_2) and four carbohydrate binding R-modules (R_1-R_4) (Figure 1) [2]. Previous studies show that A_1 introduces G to both polyMG and polyM, while A_2 creates MG-blocks from polyM, when the A-modules and their subsequent R-modules are expressed separately [2]. The present study seeks to better understand how the modules function together, and how they influence substrate binding and enzyme processivity. The main methods used were mutational studies combined with NMR spectroscopy.

This study consists of three parts. In the first part of the study, the two A-modules were inactivated, which confirmed the specificity of the A-modules. To enable mutation, a small three amino acid residue motif was inserted in the beginning of A_2, which disrupted their processiveness and substrate binding.

The second part of the study investigated what happens when the relative position of A_1 and A_2 are interchanged, and when A_2R_4 are switched to the N-terminal of the enzyme. Both modifications changed the mode of action of AlgE1.

Lastly, the A-modules of AlgE1 were replaced with the A-module of AlgE7, which is similar in structure, but performs both epimerisation and lyase reactions. This showed that AlgE1 processes along the alginate chain with the C-terminal end first (see Figure 1).

Overall, the study widens the understanding of the role of the two catalytic modules of AlgE1 and how they function together.

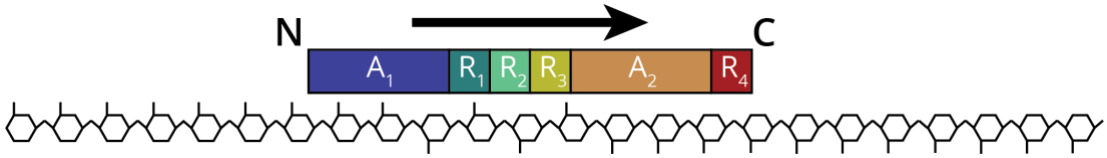


Figure 1. Modular structure of AlgE1 and its processive reaction with alginate

References:

1. Petersen AB, Tøndervik A, Gaardløs M, Ertesvåg H, Sletta H, and Aachmann FL (2023) Mannuronate C-5 Epimerases and Their Use in Alginate Modification. *Essays in Biochemistry*, 67(3): 615-627.
2. Ertesvåg H, Høidal HK, Skjåk-Bræk G, and Valla S (1998) The *Azotobacter vinelandii* Mannuronan C-5-Epimerase AlgE1 Consists of Two Separate Catalytic Domains. *Journal of Biological Chemistry*, 273(47) 30927-30932

Discovery and characterization of CRISPR-Cas endonucleases from cold-adapted bacteria

Greta Daae Sandsdalenl[1], Maryam Imam[2], Ole Morten Seternes[3], Adele Williamson[4] & Hanna-Kirsti Schrøder Leiros[1]

[1]Biomolecular and Structural Chemistry, Department of Chemistry, UiT The Arctic University of Norway, [2]The Norwegian College of Fishery Science, UiT The Arctic University of Norway, [3]Department of Phamacy, UiT The Arctic University of Norway, [4]The University of Waikato, New Zealand

The CRISPR-Cas genome editing system has revolutionized molecular biology, providing an array of biotechnological tools for carrying out precision genome modification and regulation. One limitation of the system at present is that most available tools are developed from and optimized for mesophilic organisms, which limits their utility in cold living organisms. This paucity of available knowledge on psychrophilic CRISPR-Cas systems and affiliated genome editing tools is particularly problematic for researchers of cold-blooded eukaryotes, where mismatched thermal preferences of the CRISPR components hinder application efficiency.

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 main goal is to discover and develop one or more CRISPR-associated endonucleases for efficient and precise genome editing at low temperatures. A second aim is to gain insights on CRISPR-systems across cold-adapted bacteria through bioinformatics analysis.

Our findings reveal a low prevalence of CRISPR-Cas systems in cold-adapted bacteria, compared to mesophilic and thermophilic species, where only 17.7% of the analyzed genomes contained CRISPR operons. Further, five CRISPR endonucleases were selected for experimental characterization. Currently, one of them shows promise for genome editing applications in low-temperature conditions.

The identification and initial characterization of Cas endonucleases from cold-adapted bacteria mark a pivotal step towards establishing a CRISPR-Cas platform optimized for salmonids. This advancement could significantly impact genomic studies and biotechnological applications for cold-adapted organisms, aligning with the goals of enhancing salmon health and aquaculture sustainability.

Structural insights into the interaction of Chromosomal Passenger Complex and Protein Phosphatase 2A with Shugoshin 1

Hemanga Gogoi, Dario Segura-Peña & Nikolina Sekulić

Centre for Molecular Medicine Norway, University of Oslo

The chromosomal passenger complex (CPC) is a macromolecular assembly comprising four subunits: Aurora B kinase, Inner Centromere Protein (INCENP), Survivin, and Borealin. This complex plays a role in the regulation of chromosomal segregation during mitosis. Additionally, Shugoshin 1 (Sgo1) ensures chromosome stability as it protects the cohesin, through recruitment of two protein complexes with opposite enzymatic activities, the Protein phosphatase 2A (PP2A) and the CPC. The enzymatic component of the CPC is the serine/threonine protein kinase Aurora B. Localization of Aurora B to the inner centromere is critical for proper chromosome segregation and therefore chromosome stability. Sgo1, plays an important role in the recruitment of the CPC to the inner centromere but the biochemical and structural details of the Sgo-CPC interaction are not understood. Here we undertake a biochemical and structural approach to understand this critical interaction, base in Hydrogen deuterium exchange, mutagenesis, and Fida-Bio experiments, we found that borealin is the subunit of the CPC that is more engaged in interaction with Sgo1. In addition, we show in-vitro that both the CPC and PP2A can interact simultaneously with Sgo1 and propose a model for the formation of this ternary complex.

Enzyme display on *Bacillus subtilis* spores

Jan Benedict Spannenkrebs, Agnes Beenfeldt Petersen & Prof. Johannes Kabisch

NTNU, Institute for Biotechnology and Food Science
jan.b.spannenkrebs@ntnu.no

Bacillus subtilis can form metabolically dormant endospores in response to deteriorating environmental conditions such as poor nutrient availability. In a process called sporulation, a *Bacillus subtilis* cell divides asymmetrically. In one half of the cell a copy of the DNA is enclosed in multiple membrane and proteinaceous layers. Once sporulation concludes, the mother cell lyses, releasing the metabolically dormant spore into the environment. The formed spore provides protection for the DNA against extreme / harmful conditions, including high temperatures, desiccation, radiation and irritating chemicals. The spore core, which contains the DNA, is encased in an inner and outer coat. The outermost layer, designated the "crust," is primarily composed of six proteins (CotV, W, X, Y, Z, and CgeA)[1].

These crust proteins as well as others from different spore layers have already been used to create fusion proteins with proteins of interest, for example enzymes or antigens. For this, a copy of the fusion proteins DNA is inserted into *B. subtilis* under the control of a sporulation specific promoter. These fusion proteins self-assemble into the respective spore layer during sporulation. This system has for example been used for the display of a photodecarboxylase for the transformation of lipids to hydrocarbons[2], as well as for the display of the receptor binding domain of SARS-CoV-2[3]. The immobilization of proteins in this manner greatly facilitates downstream purification. Due to the spores' size, purification is possible by comparatively low-tech means like repeated centrifugation and washing.

A novel candidate class of enzymes for spore display are Alginate epimerases such as the processive AlgE-type. Alginates are linear polysaccharides comprised of linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G). The G-blocks can chelate divalent cations like Ca^{2+}, resulting in a hydrogel. The properties of the hydrogel can be modified by altering the ratio and sequence of the M and G blocks, making them an interesting product for example the food and pharmaceutical industry[4]. Alginate epimerases are enzymes capable of epimerizing M into G blocks, thus modifying the alginates properties.

References:

1. Bartels, J., Blüher, A., López Castellanos, S., Richter, M., Günther, M. and Mascher, T. (2019), The *Bacillus subtilis* endospore crust: protein interaction network, architecture and glycosylation state of a potential glycoprotein layer. Mol Microbiol, 112: 1576-1592.
2. Karava, M.; Gockel, P.; Kabisch, J. Bacillus Subtilis Spore Surface Display of Photodecarboxylase for the Transformation of Lipids to Hydrocarbons. *Sustainable Energy Fuels* **2021**, *5* (6), 1727–1733.
3. A. Vetráková, R. Kalianková Chovanová, R. Rechtoríková, D. Krajčíková, I. Barák, Bacillus subtilis spores displaying RBD domain of SARS-CoV-2 spike protein, Computational and Structural Biotechnology Journal, Volume 21, 2023, Pages 1550-making 1556.
4. Petersen AB, Tøndervik A, Gaardløs M, Ertesvåg H, Sletta H, Aachmann FL. Mannuronate C-5 Epimerases and Their Use in Alginate Modification. Essays in Biochemistry. 2023;67(3):615-27.

THURSDAY – SESSION 2 (kl. 10.00)

3-MINUTE FLASH PRESENTATIONS

10-MINUTE STANDARD PRESENTATIONS

Nanobodies: accelerating progress in protein science and interdisciplinary research

José M. Godoy Muñoz[1] & Petri Kursula[1,2]

[1]Department of Biomedicine, University of Bergen, Bergen, Norway, [2]Faculty of Biochemistry and Molecular Medicine & Biocenter Oulu, University of Oulu, Oulu, Finland

Nanobodies, or single-domain antibodies, are emerging as state-of-the-art molecular tools for protein science. Their small size and high antigen specificity, among other characteristics, make them versatile tools to study the structure and function of their target proteins. A testament to this comes with the recent characterisation of two nanobodies targeting the activity-regulated cytoskeleton-associated protein (Arc), a complex regulator of synaptic plasticity.

These two nanobodies, E5 and H11, acted as crystallisation chaperones and promoted the structural characterization of the human Arc N-lobe (Arc-NL) at atomic resolution via X-ray crystallography. Both nanobodies bound the multi-peptide binding site of Arc-NL and inhibited the binding of a high-affinity endogenous peptide, as demonstrated using isothermal titration calorimetry. In addition, sequence homology searches of the nanobodies' CDR3s revealed new potential binding partners of Arc-NL and helped identify the possible binding site of a well-known Arc binding partner (PICK1).

These results are only a fraction of the vast potential of nanobodies. Currently, nanobodies against other proteins of the nervous system have been developed. Our present focus is to extend the use of nanobodies to immunohistochemistry and explore their potential within interdisciplinary research, putting special emphasis on brain myelination.

Giant viruses from the arctic

Juliana Miranda Tatara[1], Victoria Queiroz[2],Klara Stensvåg[1], Jônatas Abrahão[2] & Gabriel Magno De Almeida[1]

[1]The Arctic University of Norway, Tromsø, Norway, [2]Universidade Federal de Minas Gerais, Brazil

Giant viruses are part of the nucleocytoplasmic large DNA viruses (NCLDVs) group, and they were first described in 2003 [1], leading to a revolution in microbiology. Recent metagenomic research revealed that giant viruses are ubiquitous [2], and their genome size can reach up to 2.5Mb [3]. Such characteristics lead to a remarkable biotechnology potential coded by their still mysterious genomes. The arctic region, such as the Nordic Sea, have been described as hotspots for finding giant viruses [4]. Herein, we aimed to collect different samples above the arctic circle and use them to isolate giant viruses using Acanthamoeba sp. as hosts, and further explore different compounds produced during infection using one of our isolates as model. So far, we collected over 250 samples around the north of Norway and Nordic Sea, including: marine and freshwater environments, urban, sewage and deep-sea vent samples. All of them were prepared and used for virus isolation by mixing samples and cells, following culturing steps until cytopathic effect (CPE) appeared. Six different samples presented CPE and the presence of a giant virus was confirmed by Transmission Electron Microscopy images. Virus species are still to be confirmed through sequencing analysis, yet morphological features suggest viruses to be part of the marseilleviridae and mimiviridae families. Preliminary results from metabolomics analysis pointed to different compounds being produced during a Norwegian marseillevirus infection in amoeba. Subsequent steps are virus species identification and characterization, and the description of these diverse compounds produced during the infection.

References:

1. La Scola, Bernard et al. “A giant virus in amoebae.” Science (New York, N.Y.) vol. 299,5615 (2003): 2033. doi:10.1126/science.1081867.
2. Schulz, Frederik et al. “Giant virus diversity and host interactions through global metagenomics.” Nature vol. 578,7795 (2020): 432-436. doi:10.1038/s41586-020-1957-x.
3. Philippe, Nadège et al. “Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes.” Science (New York, N.Y.) vol. 341,6143 (2013): 281-6. doi:10.1126/science.1239181.
4. Gao, Chen et al. “Viral Characteristics of the Warm Atlantic and Cold Arctic Water Masses in the Nordic Seas.” Applied and environmental microbiology vol. 87,22 (2021): e0116021. doi:10.1128/AEM.01160-2.

Synthesis of dual action based antibacterial compounds

Liza Nguyen, Emil Lindbäck & Magne O. Sydnes

University of Stavanger

Bacteria have always been a threat to human health, but the hazard and mortality rates have been increased with the emergence of resistance strains.[1] Their mechanism of defence and natural evolution have compromised the effectiveness of antibiotics, to the extent that there may be no viable medicine on the market to counter bacterial infection in the future.[2]

Dual action-based molecules are one of the approaches that can be explored to design new drugs for various diseases, including cancer,[3] diabete,[4] and alzheimer.[5] In the context of bacterial resistance, several hybrid medicines have been synthesized during the past decades, as part of an effort to overcome and find a solution to the global bacterial resistance.[6]

The Marie Curie project "Stop Spread Bad Bugs" (SSBB) aims to develop and test new dual action-based molecules with antibacterial properties. The linkage of a known active scaffold (Ciprofloxacin) with new oligosaccharides offers a combination of their respective pharmaceutical activities and the novelty of the structure is making it more difficult for bacteria to develop simultaneous resistance mechanisms against both moieties.[7]

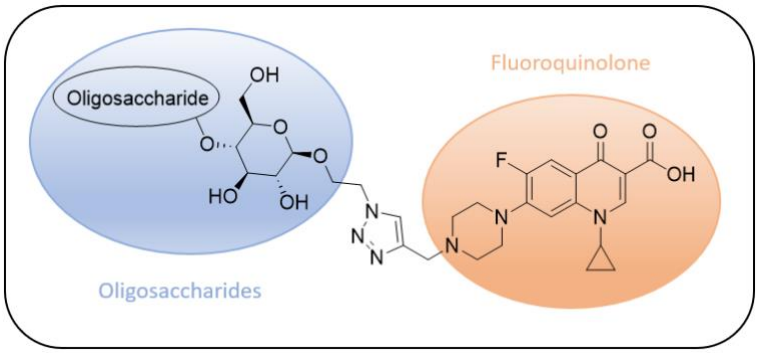


Figure 1: Target compound.

References:

1. Baindara, P.; Mandal, S. M. *Protein Pept. Lett.* **2019**, *26*, 324-331.
2. World Health Organization, *Antibacterial agents in clinical development : an analysis of the antibacterial clinical development pipeline, including tuberculosis*, **2017**.
3. Rou, R.; Ria, T.; RoyMahaPatra, D.; Hossain Sk, U. *ACS Omega*, **2023**, *19*, 16532-16544.
4. Woolston, C., *Nature*, **2013**, 14062.
5. Cheong, S.L.; Tiew, J.K.; Fong, Y.H.; Leong, H.W.; Chan, Y.M.; Chan, Z.L.; Kong, E.W.J. *Pharmaceuticals*, **2022**, *15*, 1560.
6. Bremner, J. B.; Ambrus, J. I.; Samosorn, S. *Curr. Med. Chem*., **2007**, *14*, 1459-1477.
7. Roemhild, R.; Bollenbach, T.; Andersson, D. I. *Nat. Rev. Microbiol*. **2022**, *20*, 478-490.

Bioconversion of industrial side streams from the marine sector

Maria Wilhelmsen Hoff[1], Peik Haugen[1], Terje Vasskog[1], Njål Rauø[2], Lindsey Martinsen[3], Johann Eksteen[4] & Gøril Laugsand[5]

[1]UiT Norges arktiske universitet, [2]PHARMAQ, [3]Amicoat, [4]Ard Innovation and [5]Alliance Healthcare

Sustainable technological solutions that maximize the utilization of renewable biological resources are essential to the development of the bioeconomy[1,2]. The key principle of the bioeconomy is the use of natural resources and recycling of these resources. "Green microbiology" is presented as a solution to mitigate the detrimental environmental effects seen across several large industries. These industries include food and energy production, as well as waste management. Microorganisms not only requires less amounts of natural resources for production but are easy to dispose of. A main challenge in the development of sustainable microbial processes is the cost of upscaling[3].

By combining the use of a sequential batch reactor (SBR), mainly used in wastewater treatment, with a mixed microbial community (MMC), operational costs are substantially reduced. This is largely due to removing the need for sterile conditions[4]. In addition, MMCs has the potential to utilize complex feedstocks due to the metabolic diversity. This allows for further reduction of operational costs and has the potential to increase the value of underutilized materials[5,6].

Large quantities of organic side streams with low value are produced in the aquaculture industry[4]. Simultaneously the demand for seafood increases, and with it the need for sustainable feedstocks which ensure both fish welfare and sufficient nutritional quality[7].

A challenge with MMCs is the selection time, which is often lengthy. In this project, a feast and famine regime with uncoupled carbon and nitrogen feeding was implemented for internal and external selective pressure[4,5]. The aim is to establish a stable SBR operation enriched in lipid storing bacteria for production of a bacterial meal with a lipid composition suitable as a fish feed additive.

References:

1. European Commission (2020). Bioeconomy. Bioeconomy | Research and Innovation (europa.eu)
2. Forskningsrådet, Innovasjon Norge og Siva (2019). Bioøkonomi – felles handlingsplan for forskning og innovasjon | Norges Forskingsråd (www.forskingsradet.no/publikasjoner)
3. A. A. Akinsemolu 2023, Environmental Advances 14:100440.
4. Marreios et al. 2023, Journal of Environmental Chemical Engineering, 11:110100.
5. Oliveira et al. 2017, New Biotechnology, 37(A), 69-79.
6. De Groof et al. 2019, Molecules, 24(3):398.
7. Sprague et al. 2016, 2006–2015. Scientific Reports 6:21892.

Characterization of the complex formed between phenylalanine hydroxylase and DNAJC12

Mary Dayne Sia Tai[1], Marte Innselset Flydal[2], Gloria Gamiz-Arco[1], Trond-André Kråkenes[1], Christer Flagtvedt Didriksen[1], Juha Pekka Kallio[1] & Aurora Martinez[1]

[1]Department of Biomedicine, University of Bergen, Bergen; [2]Department of Medical Genetics, Haukeland University Hospital

Hyperphenylalaninemia (HPA) primarily results from pathogenic variants in the PAH gene, which encodes phenylalanine hydroxylase (PAH), the enzyme responsible for converting L-Phe to L-Tyr. Variants of DNAJC12 also cause hyperphenylalaninemia along with dystonia, intellectual disability and neurotransmitter deficiencies in patients without any variants in PAH, in other tetrahydrobiopterin (BH4) dependent hydroxylases or in enzymes involved in BH4 synthesis or regeneration. As an Hsp40 protein, DNAJC12 binds to its client proteins, such as PAH, and presents them to Hsp70 for proper protein folding and homeostasis. However, the mechanism by which DNAJC12 binds to PAH is currently unknown. Human DNAJC12 and PAH, wild-type (WT) and HPA-associated variants, were recombinantly expressed in E. coli and purified before in vitro complex reconstitution. Biophysical and biochemical methods such as analytical size exclusion chromatography (SEC), native PAGE, immunoblotting and dynamic light scattering (DLS) were used to confirm complex formation and investigate the effect of complex formation on the stability of PAH. DNAJC12 and PAH form a complex that can be purified for further characterization. Results from SDS-PAGE, native PAGE and immunoblotting confirm the co-migration of DNAJC12 and PAH in non-denaturing conditions. By monitoring the time-dependent self-aggregation of PAH and HPA associated variants over time using DLS, DNAJC12 was also found to significantly delay PAH aggregation in vitro. Removal of an evolutionarily conserved octapeptide sequence in DNAJC12 was found to abolish its ability to bind to PAH, indicating the significance of this motif for DNAJC12 client binding. DNAJC12 recognizes and binds PAH through an evolutionarily-conserved octapeptide sequence. The binding of DNAJC12 stabilizes PAH, preventing its self-aggregation over time.

A chemo-enzymatic method for preparation of saturated oligosaccharides from alginate and other uronic acid-containing polysaccharides

Mina Gravdahl[1], Olav A. Aarstad[1], Agnes B. Petersen[1], Stina G. Karlsen[1], Ivan Donati[2], Mirjam Czjzek[3], Ove Alexander Høgmoen Åstrand[4], Philip D. Rye[4], Anne Tøndervik[5], Håvard Sletta[5], Finn L. Aachmann[1] & Gudmund Skjåk-Bræk[1]

Norwegian Biopolymer Laboratory, Department of Biotechnology and Food Science, Norwegian University of Science and Technology, Trondheim, Norway, [2]Department of Life Sciences, University of Trieste, Trieste, Italy, [3]Statiotation Biologique de Roscoff (SBR), Sorbonne Université, Roscoff, France, [4]AlgiPharma AS, Sandvika, Norway, [5]Department of Biotechnology and Nanomedicine, SINTEF Industry, Trondheim, Norway.

Oligosaccharides from uronic acid-containing polysaccharides can be produced either by chemical or enzymatic degradation. The benefit of using enzymes, called lyases, is their high specificity for various glycosidic linkages. Lyases cleave the polysaccharide chain by an β-elimination reaction, yielding oligosaccharides with an unsaturated sugar (4-deoxy-L-*erythro*-hex-4-enepyranosyluronate) at the non-reducing end. In this work we have systematically studied acid degradation of unsaturated uronic acid oligosaccharides. Based on these findings, a method for preparing saturated oligosaccharides by enzymatic degradation of uronic acid-containing polysaccharides was developed. This results in oligosaccharides with a pre-defined distribution and proportion of sugar residues compared to the products of chemical degradation, while maintaining the chemical structure of the non-reducing end. The described method was demonstrated for generating saturated oligosaccharides of alginate, heparin and polygalacturonic acid. In the case of alginate, the ratio of hydrolysis rate of Δ-G and Δ-M linkages to that of G-G and M-M linkages, respectively, was found to be approximately 65 and 43, at pH* 3.4, 90 ˚C. Finally, this method has been demonstrated to be superior in the production of α-L-guluronate oligosaccharides with a lower content of β-D-mannuronate residues compared to what can be achieved using chemical depolymerization alone.

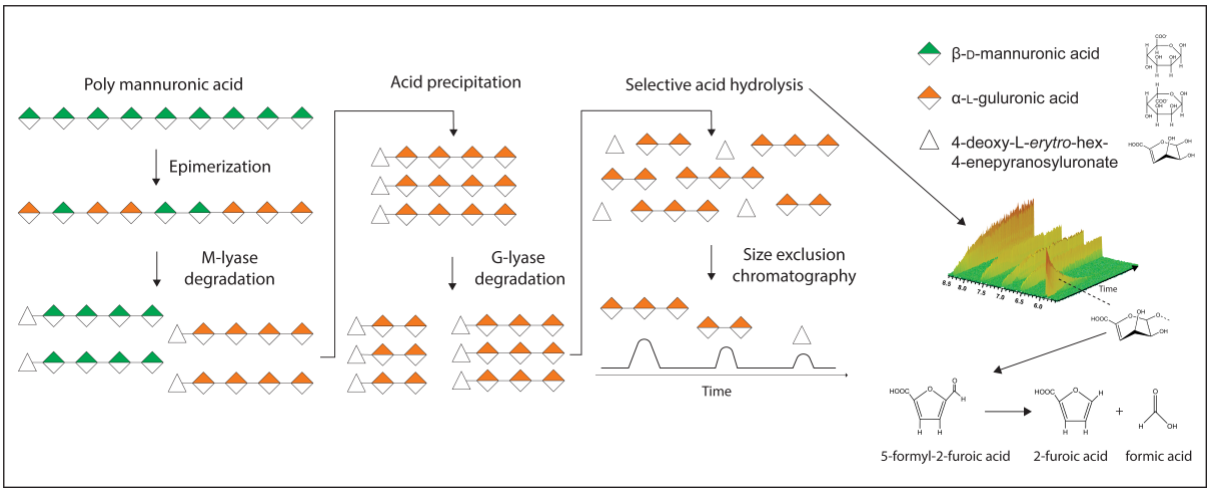


Figure 1. Graphical abstract of the chemo-enzymatic method performed on alginate.

Ligand binding to enzymes is a “dance” of mutual adaptation: implications for peptidic drug design

Robin Jeske and Richard A. Engh

Department of Chemistry (NORSTRUCT), UiT – The Arctic University of Norway, Tromsø

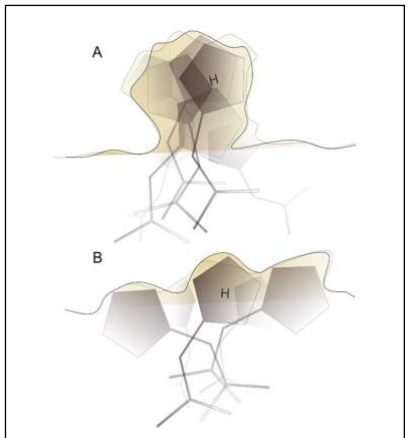
The thermodynamics and kinetics of ligand (L) binding to enzymes (E) in biophysical studies and drug design applications is typically characterized assuming a simple association reaction scheme:

$$E + L \rightleftharpoons EL$$

which is associated with a Gibbs energy of binding (ΔG_{bind}), an equilibrium constant, and on and off rates (k_{on}, k_{off}) of binding. This may be misleading in a biological context, as it obscures important physical properties of the binding process, including especially structural multiplicity or disorder and the diversity of chemical environments in molecularly crowded spaces. A more complete scheme may be abbreviated as:

$$\{E\} + \{L\} \rightleftharpoons \{E\}\{L\} \rightleftharpoons \{EL\}^{\#} \rightleftharpoons \{EL\}$$

Here, the brackets indicate sets of differing structures whose statistical distributions depend on the changing biological environment, including the proximity of the binding partner. The “#” sign marks a set of higher energy transition state type complexes, and the lower energy final bound state may also be a set of structural conformations. Binding involves a trajectory across a total energy landscape that encodes the process into a reciprocal dance of the binding partners (with their individual average monomeric energy landscapes modulated by proximities of each other, and of other species on the molecularly crowded “dance floor” of the biological environment).



Schematic of binding entropy

The disorder corresponds to the entropy of the specific averaged states along the reaction coordinate. The figure at right illustrates the entropies of rapidly interconverting structures of a single state (A) and of more distinct structural states, likely with slower interconversion rates (B), and likely with lowered enthalpy. Detailed structural and biophysical studies are needed to characterize entropy across the reaction coordinate.

This has practical implications for peptidic drug design, important for our efforts to design peptide ligands of DYRK1A and other protein kinases. Because peptides are highly flexible compared to typical small molecules, entropy considerations are especially important. Prioritizing compounds that modelling programs predict to have the best binding enthalpies may not be the best approach. We hypothesize that compounds predicted to have multiple good binding poses should be given special weight. Such compounds have lower entropic penalties of binding, but would also have potentially faster on-rates and also would be less susceptible to errors introduced by the simulation method.

THURSDAY – SESSION 3 (kl. 15.00)

3-MINUTE FLASH PRESENTATIONS

1. **Differential cultivability of *S. aureus* sub-populations in different growth environments: exploring phenotypic heterogeneity and growth diversity in isogenic bacterial populations**
   - **NADIA AFTAB** **PAGE 19**
2. **Molecular insights into the differential toxicity of cholera toxin and heat-labile enterotoxin**
   - **NATALIA MOJICA** **PAGE 20**
3. **Co-cultivation of engineered *Corynebacterium glutamicum* strains for efficient use of seaweed-derived substrates**
   - **IDA MARIE STEPHANSEN** **PAGE 21**

10-MINUTE STANDARD PRESENTATIONS

1. **Exploring the dynamics of carbohydrate-binding modules using NMR**
   - **SUSANNE HANSEN TROØYEN** **PAGE 22**
2. **Micelles with dual amorphous-crystalline cores formed by self-assembly of monodisperse PEG-peptoid conjugates**
   - **SZYMON SZOSTAK** **PAGE 23**
3. **On the road to the molecular microscope for intrinsically disordered proteins**
   - **TOBIAS RINDFLEISCH** **PAGE 24**
4. **Improving quality and drug delivery efficiency in hydrogels for treatment of neuropathic chronic pain**
   - **ZUZANNA SAMOL** **PAGE 25**

Differential Cultivability of *S. aureus* sub-populations in different growth environments

Exploring Phenotypic heterogeneity and growth diversity in isogenic bacterial populations

Nadia Aftab, Jonathan Hira, Bhupender Singh, Johanne U. Ericson, Mona Johannessen & Christian S. Lentz

Research Group for Host-Microbe Interaction, Department of Medical Biology, UiT – The Arctic University of Norway, Tromsø, Norway

Bacterial pathogens have evolved a variety of adaptation strategies to thrive in ever changing environment (1). Phenotypic heterogeneity, often acts as a short-term adaptive mechanism, enabling an isogenic population to cope better with environmental challenges (2). An example of this is the presence of distinct sub-populations with varying ability to adapt and grow in new milieu where some bacteria can flourish in novel nutritional conditions, while others cannot (3). This variability in growth is a critical aspect of bacterial physiology, playing a vital role in their ability to colonize diverse niches within a host environment. Given this background, we hypothesize that by cultivating isogenic bacteria under different growth conditions, we can identify and quantify the sub-populations that vary in their adaptability. Therefore, we established a simplistic invitro model that led to systematically study and characterize these growth phenotypes in *Staphylococcus aureus*, an important human pathogen. Our results showed a great deal of variability across different sub-population within a clonal population. Further investigations are underway to identify the molecular factors contributing to these distinct phenotypes and to explore their mechanisms of action, aiming to enhance our understanding of this adaptive phenomenon.

References:

1. Martino ME, Joncour P, Leenay R, Gervais H, Shah M, Hughes S, et al. Bacterial Adaptation to the Host's Diet Is a Key Evolutionary Force Shaping Drosophila-Lactobacillus Symbiosis. Cell Host Microbe. 2018;24(1):109-19.e6.
2. Kundu K, Weber N, Griebler C, Elsner M. Phenotypic heterogeneity as key factor for growth and survival under oligotrophic conditions. Environmental Microbiology. 2020;22(8):3339-56.
3. Reyes Ruiz LM, Williams CL, Tamayo R. Enhancing bacterial survival through phenotypic heterogeneity. PLoS Pathog. 2020;16(5):e1008439.

Molecular insights into the differential toxicity of cholera toxin and heat-labile enterotoxin

Natalia Mojica[1], Flore Kersten[1,2], Albert Serrano[3], Joel B. Heim[1], Gabriele Cordara[1], Ken Teter[3] & Ute Krengel[1]

[1]Department of Chemistry, University of Oslo, [2]Centre for Molecular Medicine Norway (NCMM), University of Oslo, [3]Burnett School of Biomedical Sciences, University of Central Florida

Cholera toxin (CT) and heat-labile enterotoxin (LT) are two similar AB_5 toxins responsible for the diarrhea characteristic of *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) infections. They consist of a catalytically active A1 subunit, an A2 linker, and a pentamer of cell-binding B-subunits [1]. Both toxins bind to the same GM1 surface receptor on the host cells and have similar levels of enzymatic activity, yet CT is more potent than LT, making cholera the more severe disease. The difference in toxicity has been attributed to structural differences near the C-terminus of the A2 linker (amino acid residues 226-236) [2], but the underlying molecular mechanism remains unknown. Recently, we showed that toxin disassembly by protein disulfide isomerase (PDI), which is a key event in the intoxication process, is more efficient for CT than for LT[3]. We hypothesized that the difference in toxin disassembly is related to the positioning of the A1 subunit relative to the B-pentamer[3] (Figure 1).

Here, we determined the crystal structures of two cholera toxin variants, where either one (D229E) or four (D229E, I230V, T232I, H233Y) amino acid residues in the critical A2 linker sequence were substituted for the residues present in LTA2 (Figure 1; colored residues within the pores of the grey pentamers). The results of this structural analysis will be presented here.

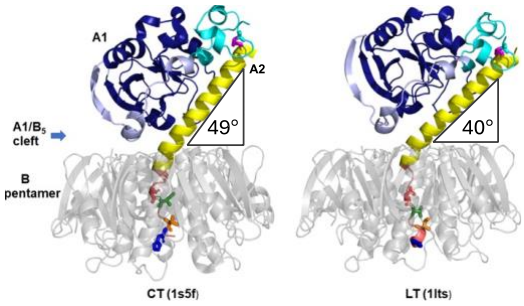


Figure 1. Structures of wild-type CT and LT [3]. For the two toxins, the angle of the A1 subunit relative to the B-pentamer differs by 9 degrees.

References:

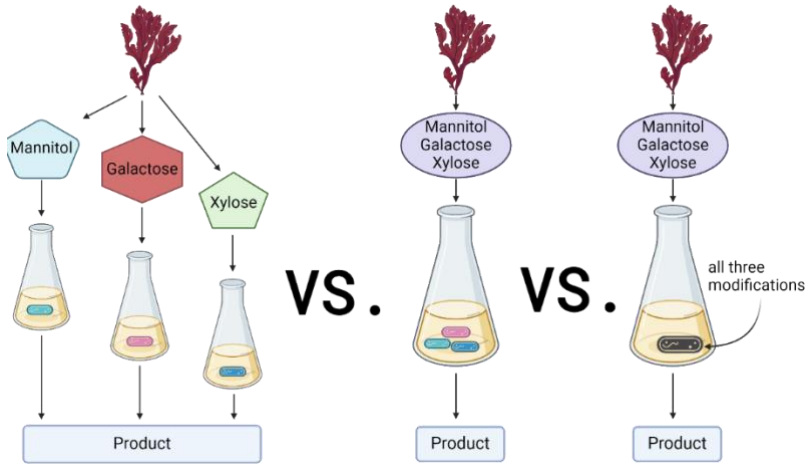
1. Heggelund, J. E., Bjørnestad, V. A., and Krengel, U. (2015) *Vibrio cholerae* and *Escherichia coli* heat-labile enterotoxins and beyond, in *The Comprehensive Sourcebook of Bacterial Protein Toxins*, pp 195–229. Elsevier Ltd.
2. Rodighiero, C., Aman, A. T., Kenny, M. J., Moss, J., Lencer, W. I., and Hirst, T. R. (1999) Structural basis for the differential toxicity of cholera toxin and *Escherichia coli* heat-labile enterotoxin. *Journal of Biological Chemistry 274*, 3962–3969.
3. Serrano, A., Guyette, J. L., Heim, J. B., Taylor, M., Cherubin, P., Krengel, U., Teter, K., and Tatulian, S. A. (2022) Holotoxin disassembly by protein disulfide isomerase is less efficient for *Escherichia coli* heat-labile enterotoxin than cholera toxin. *Sci Rep 12*, 34.

Co-cultivation of engineered *Corynebacterium glutamicum* strains for efficient use of seaweed-derived substrates

Ida Marie Stephansen[1] & Fernando Pérez-García[1*]

[1] Department of Biotechnology and Food Science, Norwegian University of Science and Technology, 7491 Trondheim, Norway
* Corresponding author: fernando.perez-garcia@ntnu.no

This project aims to meet the increasing demand for sustainable resources driven by a growing population. New renewable and alternative feedstocks are continuously sought in microbial biotechnology, and seaweed presents a promising solution. In particular, we aim to engineer the bacterium *Corynebacterium glutamicum*, which is commonly used as an industrial workhorse for the large-scale production of amino acids[1]. By applying molecular and synthetic biology tools, we will enable the utilization of seaweed-derived sugars like mannitol, xylose, or galactose by *C. glutamicum* strains[2]. Our approach will focus on co-cultivation, where each *C. glutamicum* strain will be engineered to utilize a specific sugar. We hypothesize that this method may improve the carbon conversion yield compared to classic monoculture approaches[3]. To support the concept of circular bioeconomy, utilization of seaweed sugars will be coupled with the production of added-value compounds like amino acids. Finally, to prove the potential of this idea, the newly established co-cultures will be scaled up in bioreactors using red and brown seaweed hydrolysates as the carbon source. Hence, this research seeks to contribute to sustainable production by tapping into the vast potential of ocean resources.



References:

1. Wolf, Sabrina, et al. "Advances in metabolic engineering of *Corynebacterium glutamicum* to produce high-value active ingredients for food, feed, human health, and well-being." *Essays in biochemistry* 65.2 (2021): 197-212.
2. Zhong, Haowei, et al. "The structural characteristics of seaweed polysaccharides and their application in gel drug delivery systems." *Marine drugs* 18.12 (2020): 658.
3. Pérez-García, Fernando, et al. "Dynamic co-cultivation process of *Corynebacterium glutamicum* strains for the fermentative production of riboflavin." *Fermentation* 7.1 (2021): 11.

Figure created with BioRender.com

Exploring the dynamics of carbohydrate-binding modules using NMR

Susanne Hansen Troøyen, Davide Luciano & Gaston Courtade

Department of Biotechnology and Food Science, Norwegian University of Science and Technology (NTNU). Trondheim, Norway

Several carbohydrate-active enzymes contain carbohydrate-binding modules (CBMs) that regulate enzymatic activity by localizing the catalytic domain towards the surface of insoluble substrates such as cellulose [1]. Recently, some CBMs have also been shown to have affinity for non-natural substrates such as polyethylene terephthalate (PET) [2, 3]. CBMs are thought to act as anchors on the substrate surface, allowing the enzyme to perform its activity inside a radius limited by the length of the flexible linker connecting the two domains. However, as illustrated by the Sabatier principle, the CBM should not stay attached at the same position too long – otherwise it would limit the catalytic efficiency of the enzyme. The dynamics of exchange between the free and bound state of the binding module is thus an important, but largely unexplored property of these proteins. NMR spectroscopy offers an opportunity to study protein exchange processes through carefully chosen experiments such as dark state exchange saturation transfer (DEST), solvent paramagnetic relaxation enhancement (sPRE) and relaxation rate measurements. We present here our ongoing investigation into CBM binding dynamics and identification of their substrate-binding site. In combination with affinity assays and kinetic experiments, we anticipate that these insights will contribute to developing our understanding of CBM binding mechanisms.

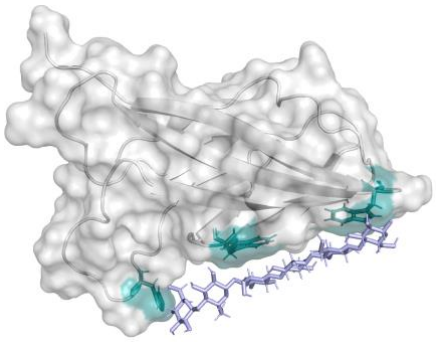


Figure 1. CBM2 from *Streptomyces coelicolor* with cellohexaose ligand.

References:

1. Courtade, G., Forsberg, Z., Heggset, E. B., et al. (2018) The carbohydrate-binding module and linker of a modular lytic polysaccharide monooxygenase promote localized cellulose oxidation. *J Biol Chem* 293(34), 13006 –13015.
2. Weber, J., Petrović, D., Strodel, B. et al. (2019) Interaction of carbohydrate-binding modules with poly(ethylene terephthalate). *Appl Microbiol Biotechnol* 103, 4801–4812.
3. Rennison, A. P., Westh, P., and Møller, M. S. (2023) Protein-plastic interactions: The driving forces behind the high affinity of a carbohydrate-binding module for polyethylene terephthalate. *Sci Total Environ* 870, 161948.

Micelles with dual amorphous-crystalline cores formed by self-assembly of monodisperse PEG-peptoid conjugates

Szymon Szostak & Reidar Lund

Department of Chemistry, University of Oslo, Norway

Peptoids (N-substituted polyglycines) are a relatively new group of synthetic polymers, synthesized for the first time in 1992,[1,2] designed to mimic peptides, while bearing some advantages like enzymatic stability or higher side chain diversity.[3] Differently from peptides, in peptoids the side chain is attached to the nitrogen atom instead of the α-carbon position in the polyglycine backbone. That small change results in the absence of backbone chirality and internal hydrogen bonding, whereas tertiary structure depends entirely on side groups' interactions.[4]

In this work, we present a new class of monodisperse poly(ethylene glycol)$_{45}$-peptoid (mPEG45-peptoid) conjugates that self-assemble into stable micelles with amorphous inner core, crystalline outer core, and diffuse PEG shell. We employed Small Angle X-ray Scattering (SAXS) with model analysis, Matrix-Assisted Laser Desorption/Ionization – Mass Spectrometry (MALDI-MS), Pendant Drop Tensiometry (PDT), and Differential Scanning Calorimetry (DSC) for detailed characterization of the compounds and self-assembled structure in biologically relevant pH range. The results give important insight into the process of sequence-dependent peptoid self-assembly showing the possibility of multi-layered nanoparticles which can be potentially used for an adjustable encapsulation system, in which the composition of the core can be altered to interact most efficiently with a desired molecule. The outer crystalline shell provides additional thermodynamic stability and an additional protective layer for the cargo and environment.

References:

1. R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, *Proc. Natl. Acad. Sci.* **1992**, 89, 9367–9371.
2. R. N. Zuckermann, *Pept. Sci.* **2011**, 96, 545–555.
3. A. Battigelli, *Biopolymers* **2019**, 110, e23265.
4. N. Gangloff, J. Ulbricht, T. Lorson, H. Schlaad, R. Luxenhofer, *Chem. Rev.* **2015**, 116, 1753–1802.

On the road to the molecular microscope for intrinsically disordered proteins

Tobias Rindfleisch[1], Jarl Underhaug[2], Hanne Antila[3] & Markus Miettinen[4]

[1]Computational Biology Unit and Department of Chemistry, University of Bergen,
[2]Department of Chemistry, University of Bergen, [3]Department of Biomedicine, University of Bergen
[4]Computational Biology Unit and Department of Chemistry, University of Bergen

Intrinsically disordered proteins (IDPs) are defined by a lack of a specific 3D structure in aqueous solution. They behave like flexible and strongly dynamic random coils, but demonstrate a characteristic distribution over the conformational space, the so-called structural ensemble. However, IDPs perform important and specific functions at the cellular level and at the super cellular level of the organism.

Molecular dynamics (MD) simulations of IDPs represent a complex problem, because none of the common IDP-specific force fields – a model in MD which defines and parameterizes the interactions of atoms – are able to describe the dynamics and thus the flexible motions of disordered proteins accurately. In consequence, the development of a force field, which is capable of reproducing the dynamics for IDPs correctly, as confirmed by validation against experimental NMR data, is of special importance.

The key-idea behind this strategy is to combine the accuracy and precision of NMR experiments with the highly intuitive visualization of MD simulations, such that NMR data can be finally interpreted via the full-atomistic representation of MD - which ultimately describes the creation of a molecular microscope.

This project is motivated by the findings of Oh et al. (2012) that dipeptides behave similarly to disordered proteins, suggesting that these elementary building blocks can be used to test and, if needed, calibrate an IDP-specific MD model.

A gradient-free evolutionary approach will be used to automatically optimize a previously selected and well performing force field; here the force field parameters are iteratively adjusted to obtain an improved match between the resulting protein dynamics in MD simulations and in NMR experiments (relaxation times, homo and hetero nuclear Nuclear Overhauser Effect). Importantly, the corresponding NMR observables can be computed from the MD simulations directly, without assuming an intervening model. The NMR experiments are performed at the Norwegian NMR Platform.

Keywords: Molecular Microscope, MD simulations, IDPs, NMR relaxation, Force field development

Corresponding Author:

Tobias Rindfleisch
PhD Student
High-Fidelity Biomolecular Modelling Group
Computational Biology Unit and Department of Chemistry, University of Bergen
tobias.rindfleisch@uib.no

Improving quality and drug delivery efficiency in hydrogels for treatment of neuropathic chronic pain

Zuzanna Samol[1], Magne O. Sydnes[2] & Erik Agner[3]

[1]Polypure AS, Norway, Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger, Norway, [2]Department of Chemistry, University of Bergen, Norway, [3]Polypure AS, Norway

Capsaicin, a well-known spicy compound found in chili peppers, binds selectively to one of the vanilloid receptors, TRPV1 (transient receptor potential vanilloid 1). When capsaicin is delivered in a high enough dose to the receptor, it can provide pain relief. Since these receptors are prevalent in sensory neurons, they are an interesting target for the treatment of chronic neuropathic pain.[1]

Capsaicin is often applied topically through creams and patches. However, these medications have several drawbacks. Creams deliver low quantities of the compound, requiring multiple reapplications throughout the day. High-concentration patches, due to their pungency and skin-burning effects, must be applied with a local anesthetic. This results in limited efficacy and patient compliance.[2,3] Therefore, there is a need to develop topical formulations with enhanced drug loading capacity and minimized side effects.

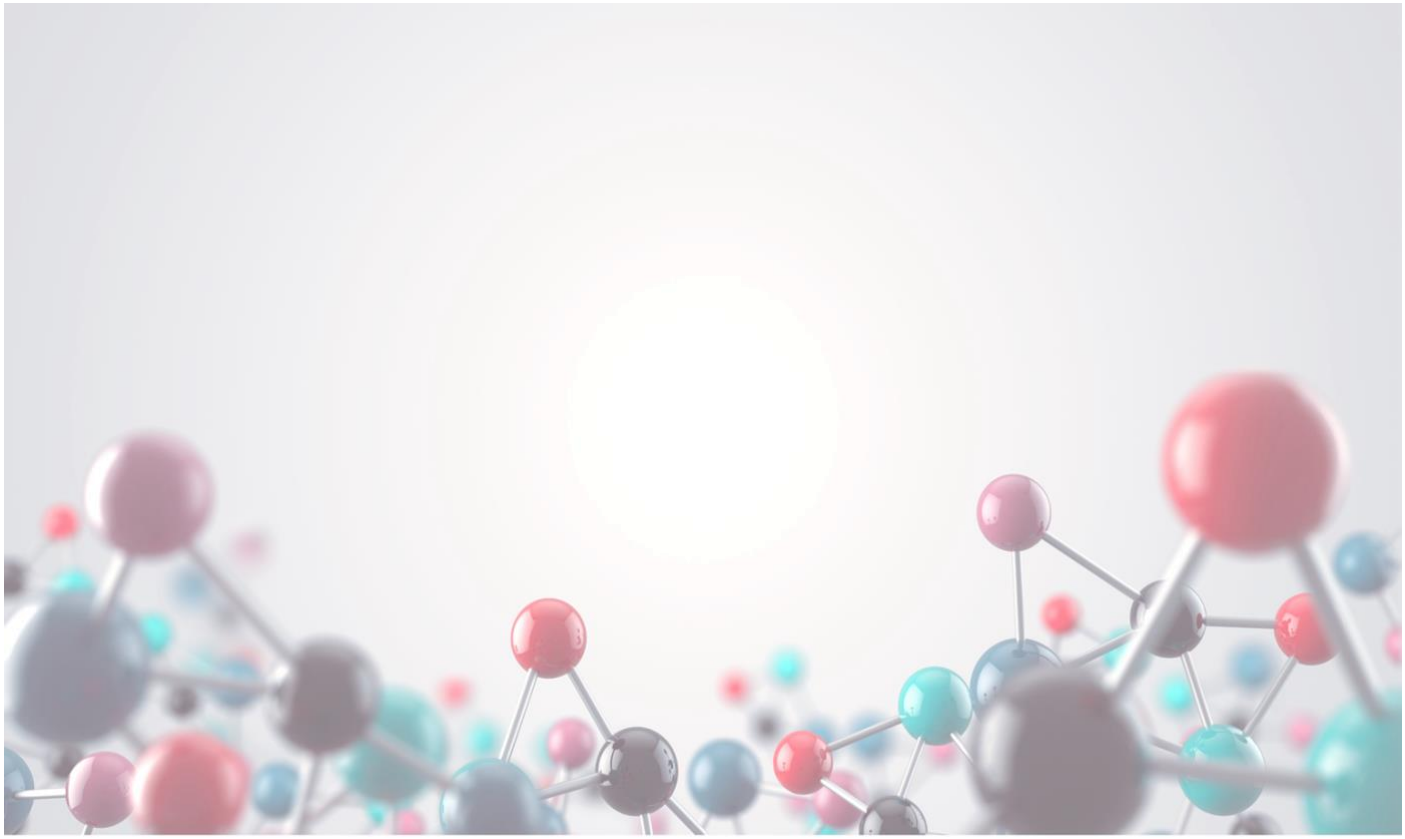
Our work focused on improving the properties of the polymeric excipients used in hydrogels for drug delivery. The employed strategies included the synthesis of monodisperse PEG and PPG derivatives, which provided defined thermosensitive copolymers. We have also investigated the effect of incorporation high-purity PPG-8 oligomer into the hydrogel. This enhanced the gelation characteristics, as well as *in vitro* release of capsaicin. In combination, these approaches offer higher purity and reproducibility of the hydrogel matrix, as well as higher hydrophobic payloads with improved delivery.

References:

1. Yang F, Zheng J. Understand spiciness: mechanism of TRPV1 channel activation by capsaicin. *Protein Cell*. 2017;8(3):169-177. doi:10.1007/s13238-016-0353-7.
2. Avila F, Torres-Guzman R, Maita K, et al. A Review on the Management of Peripheral Neuropathic Pain Following Breast Cancer. *Breast Cancer (Dove Med Press).* 2023;15:761-772. doi:10.2147/BCTT.S386803.
3. Thouaye M, Yalcin I. Neuropathic pain: From actual pharmacological treatments to new therapeutic horizons. *Pharmacol Ther*. 2023;251:108546. doi:10.1016/j.pharmthera.2023.108546.

BioCat Annual Conference

POSTER ABSTRACTS



WEDNESDAY – POSTER SESSION 1

ODD NUMBERS (kl. 19.00)

(1) Mode of Action of AlgE1 – A Modular Mannuronate C-5 Epimerase

Agnes B. Petersen[1], Anita Solem[1], Gerd Inger Sætrom[1], Håvard Sletta[2], Finn L. Aachmann[1] & Anne Tøndervik[2]

[1]Department of Biotechnology and Food Science, Norwegian University of Science and Technology. Trondheim, Norway, [2]Department of Biotechnology and Nanomedicine, SINTEF Industry. Trondheim, Norway

Alginate epimerases convert β-D-mannuronate (M) to its C-5 epimer α-L-guluronate (G) in alginates. Alginates are linear anionic polysaccharides produced by brown seaweed and some bacteria. In alginates M and G are organized in blocks of M (polyM), blocks of G (polyG), and in alternating blocks of MG (polyMG). [1]

Alginate epimerases are modular, Ca^{2+}-dependent enzymes that work in a processive manner [1] The alginate epimerase AlgE1 from *Azotobacter vinelandii* consists of two catalytically active A-modules (A_1 and A_2) and four carbohydrate binding R-modules (R_1-R_4) (Figure 1) [2]. Previous studies show that A_1 introduces G to both polyMG and polyM, while A_2 creates MG-blocks from polyM, when the A-modules and their subsequent R-modules are expressed separately [2]. The present study seeks to better understand how the modules function together, and how they influence substrate binding and enzyme processivity. The main methods used were mutational studies combined with NMR spectroscopy.

This study consists of three parts. In the first part of the study, the two A-modules were inactivated, which confirmed the specificity of the A-modules. To enable mutation, a small three amino acid residue motif was inserted in the beginning of A_2, which disrupted their processiveness and substrate binding.

The second part of the study investigated what happens when the relative position of A_1 and A_2 are interchanged, and when A_2R_4 are switched to the N-terminal of the enzyme. Both modifications changed the mode of action of AlgE1.

Lastly, the A-modules of AlgE1 were replaced with the A-module of AlgE7, which is similar in structure, but performs both epimerisation and lyase reactions. This showed that AlgE1 processes along the alginate chain with the C-terminal end first (see Figure 1).

Overall, the study widens the understanding of the role of the two catalytic modules of AlgE1 and how they function together.

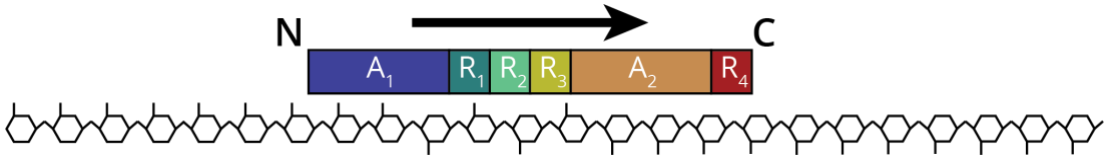


Figure 1. Modular structure of AlgE1 and its processive reaction with alginate

References:

1. Petersen AB, Tøndervik A, Gaardløs M, Ertesvåg H, Sletta H, and Aachmann FL (2023) Mannuronate C-5 Epimerases and Their Use in Alginate Modification. *Essays in Biochemistry*, 67(3): 615-627.
2. Ertesvåg H, Høidal HK, Skjåk-Bræk G, and Valla S (1998) The *Azotobacter vinelandii* Mannuronan C-5-Epimerase AlgE1 Consists of Two Separate Catalytic Domains. *Journal of Biological Chemistry*, 273(47) 30927-30932

(3) Adhesion factors and chitin derivatives – a step towards the future

Ayla Steffensen Coder, Mateu Monserrat Canals & Ute Krengel

Department of Chemistry, University of Oslo, Sem Sælands vei 26, 0371 Oslo

The pathogenic bacteria *Vibrio cholerae* cause millions of cholera infections and hundreds of thousands of recorded deaths globally each year [1]. An adhesin called N-acetylglucosamine binding protein (GbpA) is used by *V. cholerae* to both colonize the small intestines of mammals, and to adhere to chitin in plankton and shellfish [2-4]. This adhesin has lytic polysaccharide monooxygenase (LPMO) activity [3, 5], which breaks down complex carbohydrates, including chitin. GbpA's adhesive and chitinase activity enables the survival of *V.cholerae* in an aqueous environment by promoting biofilm formation and supplying the bacteria with a readily available carbon source [6]. How this protein interacts with the bacterial surface at the molecular level is poorly understood. The aim of this project is to determine the mechanism by which this adhesin binds to the bacterial surface. A combination of different methods has been and will be used, including binding assays, negative stain EM, and NMR.

A deeper understanding of the molecular mechanisms is of interest in more than one way. They may inform new treatments for cholera or other infections, and may even lead to improved ways for biofuel production from underutilized carbon sources such as chitin.

References:

1. Organization, W.H. *Cholera*. [Webpage] 2022 11 December 2023 [cited 2022 23 July 2024]; Available from: https://www.who.int/news-room/fact-sheets/detail/cholera.
2. Bhowmick, R., et al., *Intestinal Adherence of Vibrio cholerae Involves a Coordinated Interaction between Colonization Factor GbpA and Mucin.* Infection and Immunity, 2008. **76**(11): p. 4968-4977.
3. Wong, E., et al., *The Vibrio cholerae Colonization Factor GbpA Possesses a Modular Structure that Governs Binding to Different Host Surfaces.* PLOS Pathogens, 2012. **8**(1): p. e1002373.
4. Tamplin, M.L., et al., *Attachment of Vibrio cholerae serogroup O1 to zooplankton and phytoplankton of Bangladesh waters.* Appl Environ Microbiol, 1990. **56**(6): p. 1977-80.
5. Loose, J.S.M., et al., *A rapid quantitative activity assay shows that the Vibrio cholerae colonization factor GbpA is an active lytic polysaccharide monooxygenase.* FEBS Letters, 2014. **588**(18): p. 3435-3440.
6. Islam, M.S., et al., *Biofilm Acts as a Microenvironment for Plankton-Associated Vibrio cholerae in the Aquatic Environment of Bangladesh.* Microbiology and Immunology, 2007. **51**(4): p. 369-379.

(5) Computational study of Xanthan Gum Glycosyltransferase GumK

Davide Luciano[1], Jochen Schmid[2] & Gaston Courtade[1]

[1]Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, Trondheim, Norway, [2]Institute of Molecular Microbiology and Biotechnology, University of Münster, Münster, Germany

Exopolysaccharides are a diverse class of molecules with a wide range of industrial applications. Xanthan gum, a heteropolysaccharide synthesized by the Gram-negative bacterium *Xanthomonas campestris*,[1] is a significant contributor to this class of polymers. Its versatile mechanical properties make it useful in various fields such as the food, material, and pharmaceutical industries.[2] Modifications of the polysaccharide are being studied to modulate its properties for more specific applications.[3] In this study, we present a computational approach to identify key residues in the active site of GumK, a glycosyltransferase involved in xanthan biosynthesis, that influence the dynamics and activity of the enzyme. We describe the dynamics of the two domains and the residues involved in the binding of the natural substrates. Our computational protocol is applicable to other glycosyltransferases within the pathway and can elucidate enzyme selectivity. This insight can inform bioengineering strategies to produce new variants of xanthan gum.

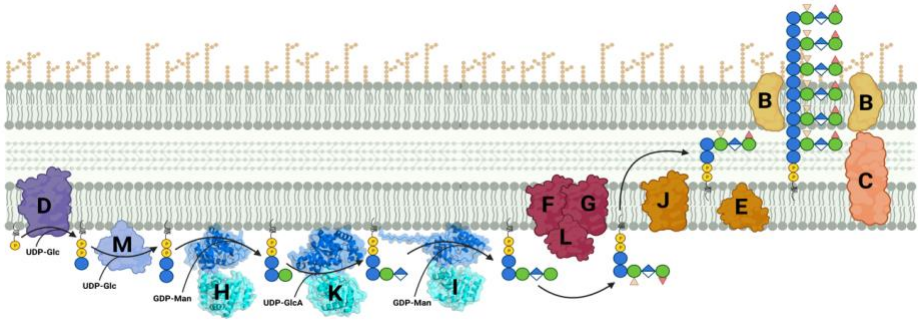


Figure 1. Set of proteins involved in the biosynthesis of xanthan gum. The target enzymes of this work are Gum H, K, I, which are involved in the assembly of the side chains of the gum

Keywords: Glycosyltransferase, GTB, Xanthan Gum, biased MD

References:

1. Anke Becker and Federico Katzen and Alfred Pühler and Luis Ielpi (1998) Xanthan gum biosynthesis and application: a biochemical /genetic perspective. *Applied Microbiology and Biotechnology*, 50: 145-152.
2. Chaturvedi Surabhi, Kulshrestha Sanchita, Bhardwaj Khushboo and Jangir Rekha (2021) A Review on Properties and Applications of Xanthan Gum. *Microbial Polymers: Applications and Ecological Perspectives*: 87-107.
3. Patel, Jwala and Maji, Biswajit and Moorthy, N. S. Hari Narayana and Maiti, Sabyasachi (2020) Xanthan gum derivatives: review of synthesis, properties and diverse applications. *RSC Adv,* 10(45): 27103-27136.

(7) Structural characterization of myelin membrane protein MAL

Gabriela Ruiz-Velarde, Valeriia Kalienkova, Andrea J. Lopez & Petri Kursula

Department of Biomedicine, University of Bergen, Jonas Lies Vei 91, 5009, Bergen, Norway

The myelin sheath is a specialized multilayer wrapped dozens of times around the axons, which is facilitated by myelin-specific proteins that participate in the stacking of the lipid membranes. One of such proteins is MAL (Myelin and Lymphocyte Protein), which is predominantly localized in the compact myelin. MAL has been associated with glycosphingolipid-rich microdomains in the myelin and other tissues, suggesting that it might play a role in the formation and maintenance of the myelin membrane.

Currently, no structural information is available for MAL, in part due to its small size. The protein measures ~17 kDa and comprises only 4 transmembrane helices which are almost entirely embedded in the membrane, making crystallization and single particle cryo-EM challenging. We aim to obtain high-resolution cryo-EM structures of MAL using membrane mimicking tools, such as nanodiscs. In addition, binding partners need to be implemented to increase the size and stability of the protein and thereby enable structure determination. Solving the structure of MAL in a lipid environment will help in better comprehending myelin formation.

We have been able to express and purify MAL from insect cells. Similar to other heterologously produced myelin proteins (PLP and DM20), MAL forms oligomeric assemblies in detergent. Furthermore, we have successfully reconstituted MAL into salipro lipid particles, and have implemented the ALFA-tag as a C-terminal extension of the last transmembrane helix. The ALFA-tag is recognized by the specific nanobody (nbALFA), which can potentially serve as a fiducial for single-particle cryo-EM. These results are a promising start for sample preparation for cryo-EM.

(9) Valorization of protein-rich poultry side-streams: spectroscopy-based monitoring of enzymatic hydrolysis for collagen content determination

Greta Nardini[1], Kristian Hovde Liland[2], Sileshi Gizachew Wubshet[3], Nils Kristian Afseth[3] & Kenneth Aase Kristoffersen[1]

[1]NMBU – Norwegian University of Life Science, KBM Faculty, P.O. Box 5003, NO-1432, Ås, Norway, [2]NMBU – Norwegian University of Life Science, REALTEK Faculty, P.O. Box 5003, NO-1433, Ås, Norway, [3]Nofima – Norwegian Institute of Food, Fisheries and Aquaculture Research, P.O. Box 210, NO-1431, Ås, Norway

Enzymatic Protein Hydrolysis (EPH) is a well-established and versatile technology for the valorization of protein from food industry side-streams. Low-value cuts from poultry, a protein-rich biomass, are transformed into high-value protein hydrolysates with different physicochemical properties. One example is collagen-enriched hydrolysates used in food products, pharmaceuticals, and cosmetics.[1]

Process monitoring and feed-forward control mechanisms are crucial to optimize yield and product quality from EPH processes.[2] The amino acid composition is an important process parameter, and along with the molecular weight distribution (MWD), it is a key parameter in determining the hydrolysates' physicochemical properties. In poultry side-stream valorization, collagen solubilization is another key parameter and can be followed using Hydroxyproline, an amino acid almost exclusive to collagen.[3]

The gold standard for amino acid composition analysis is the chromatographic method.[4] Recently, however, there has been a growing interest in spectroscopic techniques which are green, rapid, and non-destructive, making them ideal for real-time measurements.[2,5,6] Fourier Transform Infrared spectroscopy (FTIR), a vibrational spectroscopy technique, has successfully been applied to monitor industrial processes to assess relevant analytical parameters like MWD and collagen content.[7,8] While vibrational spectroscopy is not suitable for predicting the amino acid composition, Nuclear Magnetic Resonance (NMR) spectroscopy is. NMR offers deeper insights because it is superior to FTIR in probing metabolites and low molecular components, it also provides more reliable quantitative (qNMR) data.[9,10]

In this study, the performance of FTIR and NMR spectroscopy has been compared with classical methods for investigating collagen content in EPH samples from poultry side-streams.

References:

1. K. A. Kristoffersen et al., Food Chem 2022, 382, DOI 10.1016/j.foodchem.2022.132201.
2. S. G. Wubshet et al., Food Bioproc Tech 2018, 11, 2032–2043.
3. R. E. Neuman et al., Journal of Biological Chemistry 1950, 184, 299–306.
4. European Commission Regulation, No 152/2009 of 27 January 2009.
5. S. Shin et al., Food Chem 2021, 352, DOI 10.1016/j.foodchem.2021.129329.
6. M. S. Eissa et al., TrAC 2024, 170, DOI 10.1016/j.trac.2023.117435.
7. I. Måge et al., LWT 2021, 152, DOI 10.1016/j.lwt.2021.112339.

(11) Using digital holographic microscopy to monitor suspension cancer cells invading 3D matrix

Hanne Øye[1], Monica Hellesvik[1,2], Thomas Arnesen[1,2,3] & Henriette Aksnes[1]

[1]Department of biomedicine, University of Bergen, Norway, [2]Department of biological sciences, University of Bergen, Norway, [3]Department of surgery, Haukeland University Hospital, Norway

3D cell culture assays are becoming increasingly popular due to their higher resemblance to tissue environment. These provide an increased complexity compared to the growth on 2D surface and therefore allow studies of advanced cellular properties such as invasion. We report here on the use of 3D Matrigel cell preparations combined with a particular gentle and informative type of live-cell microscopy: quantitative digital holographic microscopy (DHM), here performed by a commercial software-integrated system, currently mostly used for 2D cell culture preparations. By demonstrating this compatibility, we highlight the possible time-efficient quantitative analysis obtained by using a commercial software-integrated DHM system, also for cells in a more advanced 3D culture environment. Further, we demonstrate an example making use of this advantage by performing quantitative DHM analysis of Matrigel-trapped single and clumps of suspension cells. For this, we benefited from the autofocus functionality of digital phase holographic imaging to obtain 3D information for cells migrating in a 3D environment. We demonstrate that it is possible to quantitatively measure tumorigenic properties like growth of cell clump (or spheroid) over time, as well as single-cell invasion out of cell clump and into the surrounding extracellular matrix. Overall, our findings highlight several possibilities for 3D digital holographic microscopy applications combined with 3D cell preparations, therein studies of drug response or genetic alterations on invasion capacity as well as on tumour growth and metastasis.

(13) Structural insights into the interaction of Chromosomal Passenger Complex and Protein Phosphatase 2A with Shugoshin 1

Hemanga Gogoi, Dario Segura-Peña & Nikolina Sekulić

Centre for Molecular Medicine Norway, University of Oslo

The chromosomal passenger complex (CPC) is a macromolecular assembly comprising four subunits: Aurora B kinase, Inner Centromere Protein (INCENP), Survivin, and Borealin. This complex plays a role in the regulation of chromosomal segregation during mitosis. Additionally, Shugoshin 1 (Sgo1) ensures chromosome stability as it protects the cohesin, through recruitment of two protein complexes with opposite enzymatic activities, the Protein phosphatase 2A (PP2A) and the CPC. The enzymatic component of the CPC is the serine/threonine protein kinase Aurora B. Localization of Aurora B to the inner centromere is critical for proper chromosome segregation and therefore chromosome stability. Sgo1, plays an important role in the recruitment of the CPC to the inner centromere but the biochemical and structural details of the Sgo-CPC interaction are not understood. Here we undertake a biochemical and structural approach to understand this critical interaction, base in Hydrogen deuterium exchange, mutagenesis, and Fida-Bio experiments, we found that borealin is the subunit of the CPC that is more engaged in interaction with Sgo1. In addition, we show in-vitro that both the CPC and PP2A can interact simultaneously with Sgo1 and propose a model for the formation of this ternary complex.

(15) Molecular interaction between LPS and bacterial toxin

Irene Fausk Crnic, Natalia Mojica & Ute Krengel

Department of Chemistry, University of Oslo

Cholera is an acute diarrhoeal disease caused by the bacterium *Vibrio cholerae*[1], which releases the bacterial toxin, cholera toxin (CT), inside the host intestine. The cholera toxin is similar in structure and function to the heat-labile enterotoxin (LT) from enterotoxigenic *Escherichia coli* (ETEC), which causes the less severe diarrhoea known as traveller's diarrhoea. Both toxins belong to the AB_5 family of toxins, characterized by a catalytically active A subunit and a pentamer of B subunits (CTB/LTB) important for binding to receptors on the host cell[2]. The B-pentamers also bind to other molecules, including lipopolysaccharides (LPS), which are an integral part of the outer membrane of Gram-negative bacteria. They consist of a hydrophobic part (lipid A) attached to the outer membrane, an inner and outer saccharide core, and an O-antigen[3]. LPS is also an important component of outer membrane vesicles (OMVs), which are the main delivery systems of LT toxins by ETEC[2].LT is attached to OMVs through the B-pentamer, which binds directly to LPS, but little is known about the detailed molecular interaction of LTB-LPS.

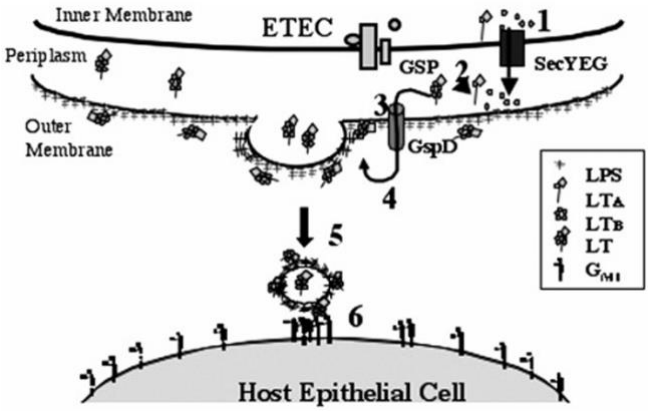


Figure 1. The delivery system of LT. Reproduced from Kuehn[1].

This project aim is to investigate the molecular interaction between the lipopolysaccharides and the B subunit of the heat-labile enterotoxin by NMR and ELISA.

References:

1. WHO. Diarrhoeal disease. World Health Organization, 2017. https://www.who.int/newsroom/fact-sheets/detail/diarrhoeal-disease (accessed 25.04.2024).
2. Horstman, A. L.; Kuehn, M. J. Bacterial surface association of heat-labile enterotoxin through lipopolysaccharide after secretion via the general secretory pathway. *J. Biol. Chem.* **2002**, *277* (36), 32538–32545. https://doi.org/10.1074/jbc.M203740200.
3. Horstman, A. L.; Bauman, S. J.; Kuehn, M. J. Lipopolysaccharide 3-deoxy-d-manno-octulosonic acid (Kdo) core determines bacterial association of secreted toxins. *J. Biol. Chem.* **2004**, *279* (9), 8070–8075. https://doi.org/10.1074/jbc.M308633200.

(17) Nanobodies targeting the human Arc N-lobe promote its crystallisation and regulate its function

José M. Godoy Muñoz[1], Lasse Neset[1], Sigurbjörn Markússon[1], Sarah Weber[1], Oda C. Krokengen[1], Aleksi Sutinen[2], Eleni Christakou[1], Andrea J. Lopez[1], Clive R. Bramham[1] & Petri Kursula[1,2]

[1]Department of Biomedicine, University of Bergen, Bergen, Norway, [2]Faculty of Biochemistry and Molecular Medicine & Biocenter Oulu, University of Oulu, Oulu, Finland

The activity-regulated cytoskeleton-associated protein (Arc) is a complex regulator of synaptic plasticity. To promote the characterisation of its function and structure, nanobodies targeting Arc have been developed. Two anti-Arc nanobodies, named E5 and H11, have been selected for further study. These nanobodies selectively bind the human Arc N-lobe (Arc-NL), which contains a multi-peptide binding pocket that mediates multiple molecular functions of Arc.

In this study, we aimed to characterise the Arc-NL-nanobody complexes of E5 and H11 from structural and functional perspectives. The structures of the complexes were solved at atomic resolution using X-ray crystallography. Interestingly, both nanobodies bind the multi-peptide binding pocket of Arc-NL. As shown by isothermal titration calorimetry, the nanobodies bind Arc-NL with nanomolar affinity and displace a peptide derived from TARPγ2, the endogenous substrate of Arc with the highest known affinity. Altogether, these results suggest that E5 and H11 can be competitive inhibitors of Arc-NL.

We have provided a thorough biochemical characterisation of E5 and H11. Both nanobodies could be used to target Arc-dependent synaptic plasticity and support the development of diagnostic and treatment tools for Arc-related disorders.

(19) Giant viruses from the arctic

Juliana Miranda Tatara[1], Victoria Queiroz[2],Klara Stensvåg[1], Jônatas Abrahão[2] & Gabriel Magno De Almeida[1]

[1]The Arctic University of Norway, Tromsø, Norway, [2]Universidade Federal de Minas Gerais, Brazil

Giant viruses are part of the nucleocytoplasmic large DNA viruses (NCLDVs) group, and they were first described in 2003 [1], leading to a revolution in microbiology. Recent metagenomic research revealed that giant viruses are ubiquitous [2], and their genome size can reach up to 2.5Mb [3]. Such characteristics lead to a remarkable biotechnology potential coded by their still mysterious genomes. The arctic region, such as the Nordic Sea, have been described as hotspots for finding giant viruses [4]. Herein, we aimed to collect different samples above the arctic circle and use them to isolate giant viruses using Acanthamoeba sp. as hosts, and further explore different compounds produced during infection using one of our isolates as model. So far, we collected over 250 samples around the north of Norway and Nordic Sea, including: marine and freshwater environments, urban, sewage and deep-sea vent samples. All of them were prepared and used for virus isolation by mixing samples and cells, following culturing steps until cytopathic effect (CPE) appeared. Six different samples presented CPE and the presence of a giant virus was confirmed by Transmission Electron Microscopy images. Virus species are still to be confirmed through sequencing analysis, yet morphological features suggest viruses to be part of the marseilleviridae and mimiviridae families. Preliminary results from metabolomics analysis pointed to different compounds being produced during a Norwegian marseillevirus infection in amoeba. Subsequent steps are virus species identification and characterization, and the description of these diverse compounds produced during the infection.

References:

1. La Scola, Bernard et al. “A giant virus in amoebae.” Science (New York, N.Y.) vol. 299,5615 (2003): 2033. doi:10.1126/science.1081867.
2. Schulz, Frederik et al. “Giant virus diversity and host interactions through global metagenomics.” Nature vol. 578,7795 (2020): 432-436. doi:10.1038/s41586-020-1957-x.
3. Philippe, Nadège et al. “Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes.” Science (New York, N.Y.) vol. 341,6143 (2013): 281-6. doi:10.1126/science.1239181.
4. Gao, Chen et al. “Viral Characteristics of the Warm Atlantic and Cold Arctic Water Masses in the Nordic Seas.” Applied and environmental microbiology vol. 87,22 (2021): e0116021. doi:10.1128/AEM.01160-2.

(21) Synthesis of dual action based antibacterial compounds

Liza Nguyen, Emil Lindbäck & Magne O. Sydnes

University of Stavanger

Bacteria have always been a threat to human health, but the hazard and mortality rates have been increased with the emergence of resistance strains.[1] Their mechanism of defence and natural evolution have compromised the effectiveness of antibiotics, to the extent that there may be no viable medicine on the market to counter bacterial infection in the future.[2]

Dual action-based molecules are one of the approaches that can be explored to design new drugs for various diseases, including cancer,[3] diabete,[4] and alzheimer.[5] In the context of bacterial resistance, several hybrid medicines have been synthesized during the past decades, as part of an effort to overcome and find a solution to the global bacterial resistance.[6]

The Marie Curie project "Stop Spread Bad Bugs" (SSBB) aims to develop and test new dual action-based molecules with antibacterial properties. The linkage of a known active scaffold (Ciprofloxacin) with new oligosaccharides offers a combination of their respective pharmaceutical activities and the novelty of the structure is making it more difficult for bacteria to develop simultaneous resistance mechanisms against both moieties.[7]

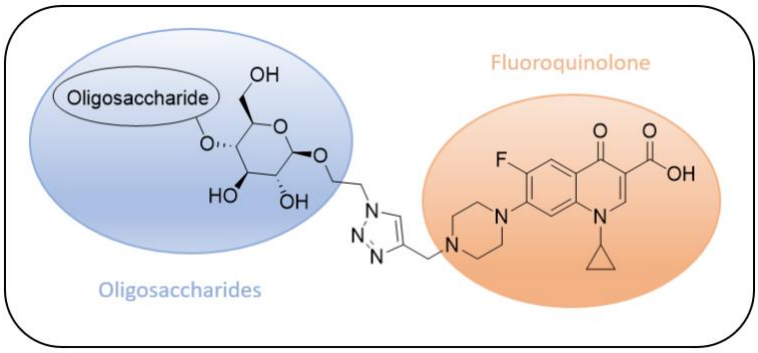


Figure 1: Target compound.

References:

1. Baindara, P.; Mandal, S. M. *Protein Pept. Lett.* **2019**, *26*, 324-331.
2. World Health Organization, *Antibacterial agents in clinical development : an analysis of the antibacterial clinical development pipeline, including tuberculosis*, **2017**.
3. Rou, R.; Ria, T.; RoyMahaPatra, D.; Hossain Sk, U. *ACS Omega*, **2023**, *19*, 16532-16544.
4. Woolston, C., *Nature*, **2013**, 14062.
5. Cheong, S.L.; Tiew, J.K.; Fong, Y.H.; Leong, H.W.; Chan, Y.M.; Chan, Z.L.; Kong, E.W.J. *Pharmaceuticals*, **2022**, *15*, 1560.
6. Bremner, J. B.; Ambrus, J. I.; Samosorn, S. *Curr. Med. Chem*., **2007**, *14*, 1459-1477.
7. Roemhild, R.; Bollenbach, T.; Andersson, D. I. *Nat. Rev. Microbiol*. **2022**, *20*, 478-490.

(23) Bioconversion of industrial side streams from the marine sector

Maria Wilhelmsen Hoff[1], Peik Haugen[1], Terje Vasskog[1], Njål Rauø[2], Lindsey Martinsen[3], Johann Eksteen[4] & Gøril Laugsand[5]

[1]UiT Norges arktiske universitet, [2]PHARMAQ, [3]Amicoat, [4]Ard Innovation and [5]Alliance Healthcare

Sustainable technological solutions that maximize the utilization of renewable biological resources are essential to the development of the bioeconomy[1,2]. The key principle of the bioeconomy is the use of natural resources and recycling of these resources. "Green microbiology" is presented as a solution to mitigate the detrimental environmental effects seen across several large industries. These industries include food and energy production, as well as waste management. Microorganisms not only requires less amounts of natural resources for production but are easy to dispose of. A main challenge in the development of sustainable microbial processes is the cost of upscaling[3].

By combining the use of a sequential batch reactor (SBR), mainly used in wastewater treatment, with a mixed microbial community (MMC), operational costs are substantially reduced. This is largely due to removing the need for sterile conditions[4]. In addition, MMCs has the potential to utilize complex feedstocks due to the metabolic diversity. This allows for further reduction of operational costs and has the potential to increase the value of underutilized materials[5,6].

Large quantities of organic side streams with low value are produced in the aquaculture industry[4]. Simultaneously the demand for seafood increases, and with it the need for sustainable feedstocks which ensure both fish welfare and sufficient nutritional quality[7].

A challenge with MMCs is the selection time, which is often lengthy. In this project, a feast and famine regime with uncoupled carbon and nitrogen feeding was implemented for internal and external selective pressure[4,5]. The aim is to establish a stable SBR operation enriched in lipid storing bacteria for production of a bacterial meal with a lipid composition suitable as a fish feed additive.

References:

1. European Commission (2020). Bioeconomy. Bioeconomy | Research and Innovation (europa.eu)
2. Forskningsrådet, Innovasjon Norge og Siva (2019). Bioøkonomi – felles handlingsplan for forskning og innovasjon | Norges Forskingsråd (www.forskingsradet.no/publikasjoner)
3. A. A. Akinsemolu 2023, Environmental Advances 14:100440.
4. Marreios et al. 2023, Journal of Environmental Chemical Engineering, 11:110100.
5. Oliveira et al. 2017, New Biotechnology, 37(A), 69-79.
6. De Groof et al. 2019, Molecules, 24(3):398.
7. Sprague et al. 2016, 2006–2015. Scientific Reports 6:21892.

(25) Calcium binding site in AA10 LPMO from *Vibrio cholerae* suggests modulating effects during environment survival and infection

Mateu Montserrat-Canals[1,2#], Kaare Bjerregaard-Andersen[2§#], Henrik Vinter Sørensen[2§], Eirik Kommedal[3M], Gabriele Cordara[2], Gustav Vaaje-Kolstad[3] & Ute Krengel[2]

[#] These authors contributed equally
[1] Centre for Molecular Medicine Norway, University of Oslo, NO-0318 Oslo, Norway
[2] Department of Chemistry, University of Oslo, NO-0315 Oslo, Norway
[3] Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), NO-1433 Ås, Norway

Despite major efforts towards its eradication, cholera remains a major health threat and economic burden in many low- and middle-income countries. Between outbreaks, the bacterium responsible for the disease, *Vibrio cholerae*, survives in aquatic environmental reservoirs, where it commonly forms biofilms, *e.g.*, on zooplankton. *N*-acetyl glucosamine binding protein A (GbpA) is an adhesin that binds to the chitinaceous surface of zooplankton and breaks its dense crystalline packing thanks to its lytic polysaccharide monooxygenase (LPMO) activity, which provides *V. cholerae* with nutrients. In addition, GbpA is an important colonization factor associated with bacterial pathogenicity, allowing the binding to mucins in the host intestine. Here, we report the discovery of a cation-binding site in proximity of the GbpA active site, which allows Ca^{2+}, Mg^{2+} or K^{+} binding close to its carbohydrate-binding surface. In addition to X-ray crystal structures of cation-LPMO complexes (to 1.5 Å resolution), we explored how the presence of ions affects the stability and activity of the protein. Calcium and magnesium ions were found to bind to GbpA specifically, with calcium ions—abundant in natural sources of chitin—having the strongest effect on protein stability. When the cation-binding site was rendered non-functional, a decrease in activity was observed, highlighting the importance of the structural elements stabilized by calcium. Our findings suggest a cation-binding site specific to GbpA and related LPMOs that may fine-tune binding and activity for the different substrates during environmental survival and host infection.

(27) A chemo-enzymatic method for preparation of saturated oligosaccharides from alginate and other uronic acid-containing polysaccharides

Mina Gravdahl[1], Olav A. Aarstad[1], Agnes B. Petersen[1], Stina G. Karlsen[1], Ivan Donati[2], Mirjam Czjzek[3], Ove Alexander Høgmoen Åstrand[4], Philip D. Rye[4], Anne Tøndervik[5], Håvard Sletta[5], Finn L. Aachmann[1] & Gudmund Skjåk-Bræk[1]

Norwegian Biopolymer Laboratory, Department of Biotechnology and Food Science, Norwegian University of Science and Technology, Trondheim, Norway, [2]Department of Life Sciences, University of Trieste, Trieste, Italy, [3]Statiotation Biologique de Roscoff (SBR), Sorbonne Université, Roscoff, France, [4]AlgiPharma AS, Sandvika, Norway, [5]Department of Biotechnology and Nanomedicine, SINTEF Industry, Trondheim, Norway.

Oligosaccharides from uronic acid-containing polysaccharides can be produced either by chemical or enzymatic degradation. The benefit of using enzymes, called lyases, is their high specificity for various glycosidic linkages. Lyases cleave the polysaccharide chain by an β-elimination reaction, yielding oligosaccharides with an unsaturated sugar (4-deoxy-L-*erythro*-hex-4-enepyranosyluronate) at the non-reducing end. In this work we have systematically studied acid degradation of unsaturated uronic acid oligosaccharides. Based on these findings, a method for preparing saturated oligosaccharides by enzymatic degradation of uronic acid-containing polysaccharides was developed. This results in oligosaccharides with a pre-defined distribution and proportion of sugar residues compared to the products of chemical degradation, while maintaining the chemical structure of the non-reducing end. The described method was demonstrated for generating saturated oligosaccharides of alginate, heparin and polygalacturonic acid. In the case of alginate, the ratio of hydrolysis rate of Δ-G and Δ-M linkages to that of G-G and M-M linkages, respectively, was found to be approximately 65 and 43, at pH* 3.4, 90 ˚C. Finally, this method has been demonstrated to be superior in the production of α-L-guluronate oligosaccharides with a lower content of β-D-mannuronate residues compared to what can be achieved using chemical depolymerization alone.

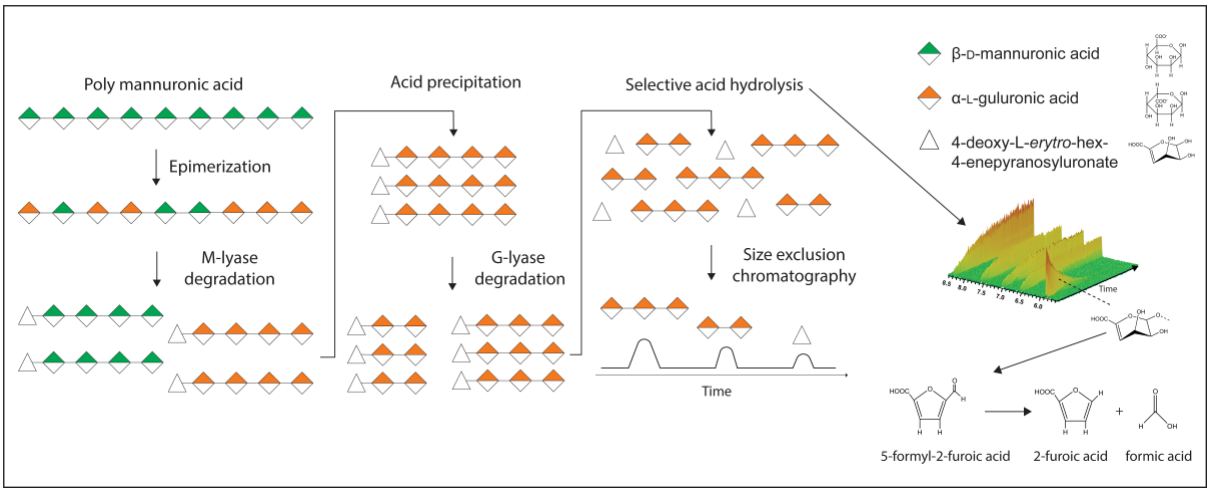


Figure 1. Graphical abstract of the chemo-enzymatic method performed on alginate.

(29) Differential Cultivability of *S. aureus* sub-populations in different growth environments

Exploring Phenotypic heterogeneity and growth diversity in isogenic bacterial populations

Nadia Aftab, Jonathan Hira, Bhupender Singh, Johanne U. Ericson, Mona Johannessen & Christian S. Lentz

Research Group for Host-Microbe Interaction, Department of Medical Biology, UiT – The Arctic University of Norway, Tromsø, Norway

Bacterial pathogens have evolved a variety of adaptation strategies to thrive in ever changing environment (1). Phenotypic heterogeneity, often acts as a short-term adaptive mechanism, enabling an isogenic population to cope better with environmental challenges (2). An example of this is the presence of distinct sub-populations with varying ability to adapt and grow in new milieu where some bacteria can flourish in novel nutritional conditions, while others cannot (3). This variability in growth is a critical aspect of bacterial physiology, playing a vital role in their ability to colonize diverse niches within a host environment. Given this background, we hypothesize that by cultivating isogenic bacteria under different growth conditions, we can identify and quantify the sub-populations that vary in their adaptability. Therefore, we established a simplistic invitro model that led to systematically study and characterize these growth phenotypes in *Staphylococcus aureus*, an important human pathogen. Our results showed a great deal of variability across different sub-population within a clonal population. Further investigations are underway to identify the molecular factors contributing to these distinct phenotypes and to explore their mechanisms of action, aiming to enhance our understanding of this adaptive phenomenon.

References:

1. Martino ME, Joncour P, Leenay R, Gervais H, Shah M, Hughes S, et al. Bacterial Adaptation to the Host's Diet Is a Key Evolutionary Force Shaping Drosophila-Lactobacillus Symbiosis. Cell Host Microbe. 2018;24(1):109-19.e6.
2. Kundu K, Weber N, Griebler C, Elsner M. Phenotypic heterogeneity as key factor for growth and survival under oligotrophic conditions. Environmental Microbiology. 2020;22(8):3339-56.
3. Reyes Ruiz LM, Williams CL, Tamayo R. Enhancing bacterial survival through phenotypic heterogeneity. PLoS Pathog. 2020;16(5):e1008439.

(31) Ligand binding to enzymes is a “dance” of mutual adaptation: implications for peptidic drug design

Robin Jeske and Richard A. Engh

Department of Chemistry (NORSTRUCT), UiT – The Arctic University of Norway, Tromsø

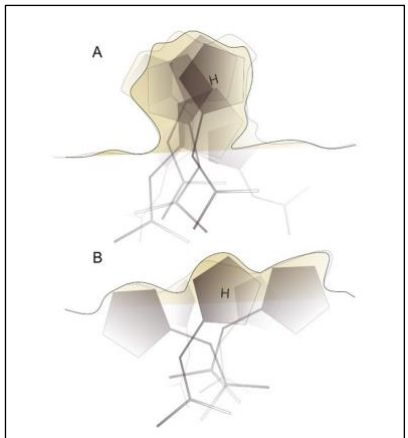
The thermodynamics and kinetics of ligand (L) binding to enzymes (E) in biophysical studies and drug design applications is typically characterized assuming a simple association reaction scheme:

$$E + L \rightleftharpoons EL$$

which is associated with a Gibbs energy of binding (ΔG_{bind}), an equilibrium constant, and on and off rates (k_{on}, k_{off}) of binding. This may be misleading in a biological context, as it obscures important physical properties of the binding process, including especially structural multiplicity or disorder and the diversity of chemical environments in molecularly crowded spaces. A more complete scheme may be abbreviated as:

$$\{E\} + \{L\} \rightleftharpoons \{E\}\{L\} \rightleftharpoons \{EL\}^{\#} \rightleftharpoons \{EL\}$$

Here, the brackets indicate sets of differing structures whose statistical distributions depend on the changing biological environment, including the proximity of the binding partner. The “#” sign marks a set of higher energy transition state type complexes, and the lower energy final bound state may also be a set of structural conformations. Binding involves a trajectory across a total energy landscape that encodes the process into a reciprocal dance of the binding partners (with their individual average monomeric energy landscapes modulated by proximities of each other, and of other species on the molecularly crowded “dance floor” of the biological environment).



Schematic of binding entropy

The disorder corresponds to the entropy of the specific averaged states along the reaction coordinate. The figure at right illustrates the entropies of rapidly interconverting structures of a single state (A) and of more distinct structural states, likely with slower interconversion rates (B), and likely with lowered enthalpy. Detailed structural and biophysical studies are needed to characterize entropy across the reaction coordinate.

This has practical implications for peptidic drug design, important for our efforts to design peptide ligands of DYRK1A and other protein kinases. Because peptides are highly flexible compared to typical small molecules, entropy considerations are especially important. Prioritizing compounds that modelling programs predict to have the best binding enthalpies may not be the best approach. We hypothesize that compounds predicted to have multiple good binding poses should be given special weight. Such compounds have lower entropic penalties of binding, but would also have potentially faster on-rates and also would be less susceptible to errors introduced by the simulation method.

(33) Tetradecylthioacetic acid analogues impact on mitochondrial function

Siri T. Sola[1], Lise Madsen[2], Marie Austdal[3], Rolf K. Berge[4] & Magne O. Sydnes[5]

[1]University of Stavanger (UiS)/Tomega AS, [2]University of Bergen(UiB), [3]Stavanger University Hospital, [4]Tomega AS,[5]UiB, UiS

Therapeutic options for mitochondrial disorders are currently limited. Consequently, there is a growing interest in discovering safe and effective approaches to address mitochondrial dysfunction. Among these, small-molecule therapies show significant promise for enhancing mitochondrial performance. (1) To prevent pathogenic processes, one approach is to use synthetic versions of mitochondrial fatty acids (MTFAs) which can be orally ingested to increase the mitochondria functioning. A structurally modified fatty acid might be resistant to both β-oxidation and ω-oxidation. One type of structurally modified fatty acid comes from Berge and his group, where the modified fatty acid, tetradecylthioacetic acid (TTA), has been synthesized and investigated (Figure 1). (2)

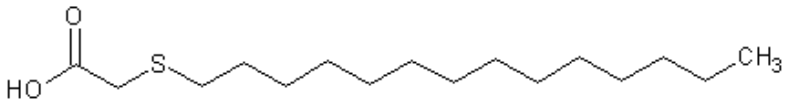


Figure 1. Tetradecylthioacetic acid (TTA). (2)

TTA was found to have a significantly positive effect on the fatty acid composition in cells. In the current project the aim is to further develop TTA to have an even greater effect on liver and brain health. By synthetic methods we have coupled TTA with another health improving molecule and tested the effect the compound in cells and in rats. (Figure 2)

Primary analysis, show that the TTA analogue effect on fatty acid composition is not significantly different from the results obtained with TTA itself (Figure 2).

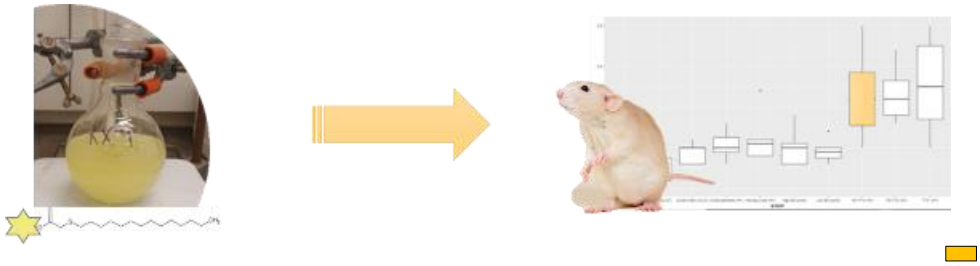


Figure 2. Rats were feed with TTA analogue for 16 weeks. Results from blood samples are analysed by R program and represented to the left.

References:

1. Meng, L.& Wu, G. (2023). Recent advances in small molecules for improving mitochondrial disorders. *RSC Advances, 13*, 20476-20485.
2. Jorgensen, M. R., Bhurruth-Alcor, Y., Rost, T., Bohov, P., Muller, M., Guisado, C., . . . Skorve, J. (2009). Synthesis and analysis of novel glycerolipids for the treatment of metabolic syndrome. *J Med Chem, 52*(4), 1172-1179.

(35) Micelles with dual amorphous-crystalline cores formed by self-assembly of monodisperse PEG-peptoid conjugates

Szymon Szostak & Reidar Lund

Department of Chemistry, University of Oslo, Norway

Peptoids (N-substituted polyglycines) are a relatively new group of synthetic polymers, synthesized for the first time in 1992,[1,2] designed to mimic peptides, while bearing some advantages like enzymatic stability or higher side chain diversity.[3] Differently from peptides, in peptoids the side chain is attached to the nitrogen atom instead of the α-carbon position in the polyglycine backbone. That small change results in the absence of backbone chirality and internal hydrogen bonding, whereas tertiary structure depends entirely on side groups' interactions.[4]

In this work, we present a new class of monodisperse poly(ethylene glycol)$_{45}$-peptoid (mPEG45-peptoid) conjugates that self-assemble into stable micelles with amorphous inner core, crystalline outer core, and diffuse PEG shell. We employed Small Angle X-ray Scattering (SAXS) with model analysis, Matrix-Assisted Laser Desorption/Ionization – Mass Spectrometry (MALDI-MS), Pendant Drop Tensiometry (PDT), and Differential Scanning Calorimetry (DSC) for detailed characterization of the compounds and self-assembled structure in biologically relevant pH range. The results give important insight into the process of sequence-dependent peptoid self-assembly showing the possibility of multi-layered nanoparticles which can be potentially used for an adjustable encapsulation system, in which the composition of the core can be altered to interact most efficiently with a desired molecule. The outer crystalline shell provides additional thermodynamic stability and an additional protective layer for the cargo and environment.

References:

1. R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, *Proc. Natl. Acad. Sci.* **1992**, 89, 9367–9371.
2. R. N. Zuckermann, *Pept. Sci.* **2011**, 96, 545–555.
3. A. Battigelli, *Biopolymers* **2019**, 110, e23265.
4. N. Gangloff, J. Ulbricht, T. Lorson, H. Schlaad, R. Luxenhofer, *Chem. Rev.* **2015**, 116, 1753–1802.

(37) On the road to the molecular microscope for intrinsically disordered proteins

Tobias Rindfleisch[1], Jarl Underhaug[2], Hanne Antila[3] & Markus Miettinen[4]

[1]Computational Biology Unit and Department of Chemistry, University of Bergen,
[2]Department of Chemistry, University of Bergen, [3]Department of Biomedicine, University of Bergen
[4]Computational Biology Unit and Department of Chemistry, University of Bergen

Intrinsically disordered proteins (IDPs) are defined by a lack of a specific 3D structure in aqueous solution. They behave like flexible and strongly dynamic random coils, but demonstrate a characteristic distribution over the conformational space, the so-called structural ensemble. However, IDPs perform important and specific functions at the cellular level and at the super cellular level of the organism.

Molecular dynamics (MD) simulations of IDPs represent a complex problem, because none of the common IDP-specific force fields – a model in MD which defines and parameterizes the interactions of atoms – are able to describe the dynamics and thus the flexible motions of disordered proteins accurately. In consequence, the development of a force field, which is capable of reproducing the dynamics for IDPs correctly, as confirmed by validation against experimental NMR data, is of special importance.

The key-idea behind this strategy is to combine the accuracy and precision of NMR experiments with the highly intuitive visualization of MD simulations, such that NMR data can be finally interpreted via the full-atomistic representation of MD - which ultimately describes the creation of a molecular microscope.

This project is motivated by the findings of Oh et al. (2012) that dipeptides behave similarly to disordered proteins, suggesting that these elementary building blocks can be used to test and, if needed, calibrate an IDP-specific MD model.

A gradient-free evolutionary approach will be used to automatically optimize a previously selected and well performing force field; here the force field parameters are iteratively adjusted to obtain an improved match between the resulting protein dynamics in MD simulations and in NMR experiments (relaxation times, homo and hetero nuclear Nuclear Overhauser Effect). Importantly, the corresponding NMR observables can be computed from the MD simulations directly, without assuming an intervening model. The NMR experiments are performed at the Norwegian NMR Platform.

Keywords: Molecular Microscope, MD simulations, IDPs, NMR relaxation, Force field development

Corresponding Author:
Tobias Rindfleisch
PhD Student
High-Fidelity Biomolecular Modelling Group
Computational Biology Unit and Department of Chemistry, University of Bergen
tobias.rindfleisch@uib.no

(39) Screening for stabilizing compounds of VMAT2 with therapeutic potential for brain monoamine vesicular transport disorders

Trond-André Kråkenes[1], Kristine Kippersund Brokstad[1], Aurora Martunez[1,2,3] & Svein Isungset Støve[1,2,3]

[1]Department of Biomedicine, University of Bergen, [2]Neuro-SysMed, Department of Neurology, Haukeland university Hospital, [3]K.G. Jebsen Center for Translational Research in Parkinson's Disease, University of Bergen

INTRODUCTION: Dopamine (DA) and other monoamine neurotransmitters are sequestered into synaptic vesicles by vesicular monoamine transporter 2 (VMAT2) at the presynaptic terminals. VMAT2 is a crucial regulator of DA homeostasis and is a potential drug target for neuronal disorders, such as Parkinson's disease and involuntary movement disorders. VMAT2 variants have been shown to cause brain monoamine vesicular transport disease, which is a severe, rare infantile-onset disorder that mimics cerebral palsy. We aim to find VMAT2 activators or stabilizers that can rescue misfolded, dysfunctional VMAT2 variants, such as P237H and P387L, and be used in the treatment of monoamine vesicular transport disease.

METHODS: Screening for VMAT2 modulators was done in HEK293 cells permanently transfected to express human VMAT2 with an mCherry fusion tag. Cells in 96 well plates were treated with compounds from the Prestwick Chemical library® (PCL; 1520 compounds, most FDA- and EMA approved) for 48 h. The inhibition or activation of VMAT2 was determined by quantifying the decreased or increased uptake of a fluorescent VMAT2 substrate in treated cells, respectively. The chaperone effect was determined by measuring the increase in protein amount by quantification of fluorescence from the mCherry fusion tag.

RESULTS: We have established a functional high-throughput cellular screening approach combining the effect of the compounds on uptake activity of a fluorescent VMAT2 substrate in cells and their effect on the stability and thus half-life of the VMAT2 in cells. Using this monitoring approach, the screening of the PCL library resulted in the identification of several novel VMAT2 modulators that will be assessed for their effect on substrate transport and protein expression level on disease causing VMAT2 variants.

CONCLUSION: We have identified small pharmacological chaperones that modulate the activity of VMAT2 in cells with a potential therapeutic effect on VMAT2 variants that cause brain monoamine vesicular transport disease.

(41) Improving quality and drug delivery efficiency in hydrogels for treatment of neuropathic chronic pain

Zuzanna Samol[1], Magne O. Sydnes[2] & Erik Agner[3]

[1]Polypure AS, Norway, Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger, Norway, [2]Department of Chemistry, University of Bergen, Norway, [3]Polypure AS, Norway

Capsaicin, a well-known spicy compound found in chili peppers, binds selectively to one of the vanilloid receptors, TRPV1 (transient receptor potential vanilloid 1). When capsaicin is delivered in a high enough dose to the receptor, it can provide pain relief. Since these receptors are prevalent in sensory neurons, they are an interesting target for the treatment of chronic neuropathic pain.[1]

Capsaicin is often applied topically through creams and patches. However, these medications have several drawbacks. Creams deliver low quantities of the compound, requiring multiple reapplications throughout the day. High-concentration patches, due to their pungency and skin-burning effects, must be applied with a local anesthetic. This results in limited efficacy and patient compliance.[2,3] Therefore, there is a need to develop topical formulations with enhanced drug loading capacity and minimized side effects.

Our work focused on improving the properties of the polymeric excipients used in hydrogels for drug delivery. The employed strategies included the synthesis of monodisperse PEG and PPG derivatives, which provided defined thermosensitive copolymers. We have also investigated the effect of incorporation high-purity PPG-8 oligomer into the hydrogel. This enhanced the gelation characteristics, as well as *in vitro* release of capsaicin. In combination, these approaches offer higher purity and reproducibility of the hydrogel matrix, as well as higher hydrophobic payloads with improved delivery.

References:

1. Yang F, Zheng J. Understand spiciness: mechanism of TRPV1 channel activation by capsaicin. *Protein Cell*. 2017;8(3):169-177. doi:10.1007/s13238-016-0353-7.
2. Avila F, Torres-Guzman R, Maita K, et al. A Review on the Management of Peripheral Neuropathic Pain Following Breast Cancer. *Breast Cancer (Dove Med Press).* 2023;15:761-772. doi:10.2147/BCTT.S386803.
3. Thouaye M, Yalcin I. Neuropathic pain: From actual pharmacological treatments to new therapeutic horizons. *Pharmacol Ther*. 2023;251:108546. doi:10.1016/j.pharmthera.2023.108546.

(43) Structural characterization of ATP-induced disassembly mechanisms in Cytolethal Distending Toxin

Ema Albrechtova, Gabriele Cordara, Ute Krengel

Department of Chemistry, University of Oslo, NO-0315 Oslo, Norway

Cytolethal distending toxins (CDTs) are a class of genotoxins produced by various Gram-negative bacteria, known for their ability to cause DNA damage and genomic instability, potentially leading to oncogenesis. CDT is a heterotrimeric AB_2 toxin consisting of a catalytic "A" subunit (CdtB) with DNase activity and two cell-binding "B" subunits (CdtA and CdtC).

As a first step to analyze the ATP-induced disassembly mechanism of CDT, we plan to study the interaction between ATP and CdtB. For this, we will use various structural biology techniques. This poster gives an introduction to our project.

The research is expected to significantly advance our understanding of bacterial toxin biology, offering potential pathways for therapeutic intervention against CDT-induced cellular damage. The insights gained from this study could lead to novel strategies for targeting CDT-related pathologies, ultimately contributing to improved clinical outcomes.

THURSDAY – POSTER SESSION 2

EVEN NUMBERS (kl. 19.00)

(2) Two ω-transaminases from the Ectoine biosynthesis and degradation pathways

Amalie Skogvold, Heidi T. Hillier & Ingar Leiros

UiT - The Arctic University of Norway, Tromsø

ω-Transaminases are biocatalysts highly sought after in the pharmaceutical industry as a green alternative for the production of valuable chiral amines. The DABA ω-transaminases EctB and DoeD catalyze the forward and reverse transaminase reactions in the bacterial ectoine biosynthesis and degradation pathways, respectively. Ectoine is a highly valuable compound used in both the cosmetics and pharmaceutical industries due to its many novel properties, such as acting as a protein stabilizer, DNA protector, and membrane, cell, and skin protectant. It is primarily produced by a method called bacterial milking, but more recent research has focused on heterologous production using non-halophilic bacteria such as *Escherichia coli*. Despite the high market demand for ectoine, research on characterizing the enzymes in the ectoine biosynthesis and degradation pathways has been limited. Recently, we solved the first crystal structure of DoeD and completed a biochemical and biophysical characterization of the transaminase, including exploring the substrate scope for other potential uses of the enzyme as a pharmaceutical biocatalyst. Previous work by the Ectoine Research Group includes solving the crystal structure of EctB from the model organism for ectoine production, *Chromohalobacter salexigens* DSM 3043 (Hillier et al., 2020). The group aims to characterize all the core enzymes involved in ectoine synthesis, including EctB, EctA, and EctC, as well as DoeD from the ectoine degradation pathway. Future work will also prioritize the rational design to improve the operational stability and efficiency of the transaminases, especially EctB and DoeD.

Reference:

1. Hillier HT et al. (2020) FEBS J 287, 4641-4658.

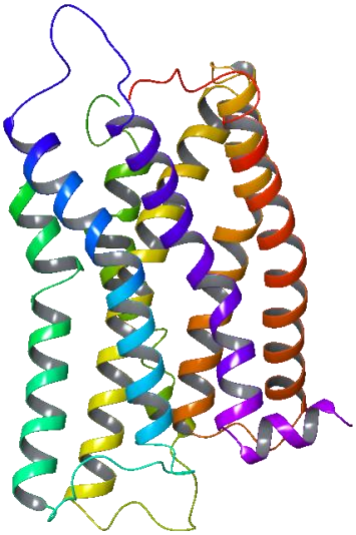
(4) Computational investigation of the binding modes of specialized pro-resolving mediators at GPR32

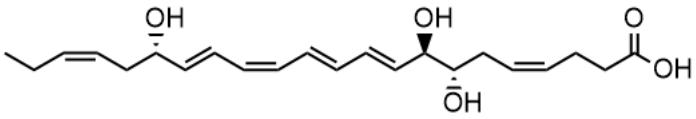
Daniel Haga Hasselstrøm, Osman Gani & Trond Vidar Hansen

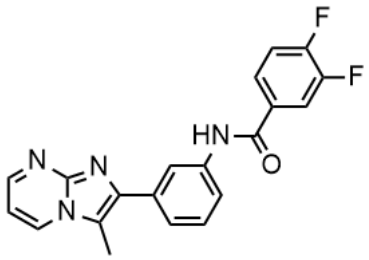
Department of Pharmacy, Section for Pharmaceutical Chemistry, University of Oslo, PO Box 1068 Blindern, N - 0316 Oslo, Norway

The G protein-coupled receptor (GPCR) GPR32 plays a crucial role in mediating the anti-inflammatory and tissue-repairing actions of several specialized pro-resolving mediators (SPMs) such as resolvin D1 and lipoxin A4, which act as potent agonists at this receptor.[1,2]

No experimental structures of GPR32 have been solved, necessitating the use of computational means to investigate the stereoselective binding of these potent fatty acid metabolites at GPR32 and synthetic agonists such as C2A.[3]







C2A

This poster presents our current work on elucidating the binding modes of resolvins and synthetic agonists at GPR32 by employing state-of-the-art computational methods such as molecular dynamics simulations, free energy calculations and residue interaction analysis.

References:

1. Krishnamoorthy, Sriram, et al. "Resolvin D1 binds human phagocytes with evidence for proresolving receptors." *Proceedings of the National Academy of Sciences* 107.4 (2010): 1660-1665.
2. Krishnamoorthy, Sriram, et al. "Resolvin D1 receptor stereoselectivity and regulation of inflammation and proresolving microRNAs." *The American journal of pathology* 180.5 (2012): 2018-2027.
3. Chiang, Nan, et al. "Identification of chemotype agonists for human resolvin D1 receptor DRV1 with pro-resolving functions." *Cell chemical biology* 26.2 (2019): 244-254.

(6) Structural modeling of antibody variant epitope specificity with complementary experimental and computational techniques

Eva Smorodina[1], Oliver Crook[2], Rahmad Akbar[1], Puneet Rawat[1], Dario Segura Pena[3], Nikolina Sekulic[3], Ole Magnus Fløgstad[3], Khang Lê Quý[1], Brij Bhushan Mehta[1], Johannes Loeffler[4], Monica Fernandez-Quintero[4], Hannah Turner[4], Andrew B. Ward[4] & Victor Greiff[1]

[1]Department of Immunology, University of Oslo and Oslo University Hospital, Oslo, Norway, [2]Department of Statistics, University of Oxford, Oxford, [3]Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, Faculty of Medicine, University of Oslo, Oslo, Norway, [4]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, USA

Antibodies are key therapeutics but the principles behind diverse paratopes binding to the same epitope remain unexplained. An insufficient understanding of the structural rules behind antibody-antigen binding, due to a lack of experimentally resolved structures, leads to the current inability to characterize antibody variants binding in silico. Here we propose a rule-based antibody design that relies on a thorough understanding of epitope-paratope interactions, in contrast to generative design based on millions of trials and errors. We identified the epitope of five affinity-verified Trastuzumab variants using cryo-EM and position-resolved HDX-MS. Rigid models alone are insufficient for accurate antibody-antigen modeling while molecular dynamics simulations with computational analysis of the complex conformations succeed in replicating and complimenting experimental findings. Structural parameters calculated based on geometry, surface, and biochemical properties were able to distinguish between high and low binders. We highlight the possibilities of AI in antibody and antibody-antigen structure modeling, demonstrating the limitations of various language-based models to predict and understand antibody variants. Overall, our study explains the binding mechanisms of the variant sequences, showing how antibodies with diverse sequences share similar antigen-binding rules.

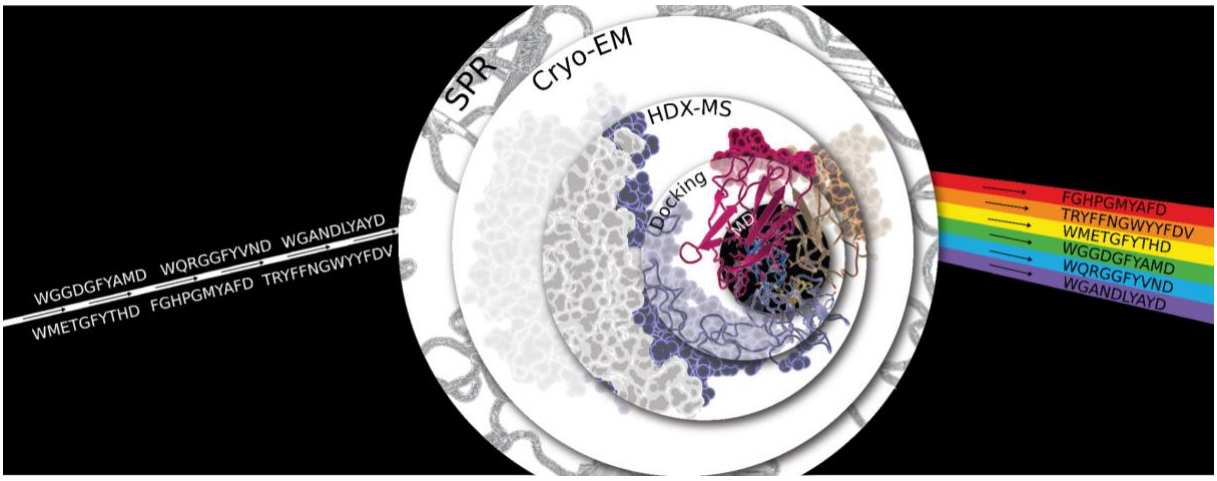


Figure 1. Different levels of complexity surrounding structural rules behind antibody-antigen binding. We start from a more general understanding of the interaction kinetics with SPR, then identify the global binding site with cryo-EM, refine the region with HDX-MS to achieve peptide-level resolution, and move forward towards residue- and atom-wise resolution with computational techniques like docking and molecular dynamics.

(8) Pharmacological chaperones as a potential therapy for Tyrosine Hydroxylase Deficiency (THD). Targeting the interaction between TH and its DNAJ Co-Chaperone

Gloria Gamiz-Arco, Mary Dayne Sia Tai, Kunwar Jung-KC & Aurora Martinez

Department of Biomedicine, University of Bergen, 5020 Bergen, Norway

Tyrosine Hydroxylase Deficiency (THD) is a rare genetic disorder characterized by severely low dopamine levels due to variants in tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of dopamine. Patients with THD present a phenotype ranging from dystonia to severe encephalopathy. Presently, the standard medication to address dopamine deficiency in these patients is the dopamine precursor levodopa (synthetic L-Dopa). While it is relatively effective in managing motor dysfunction, especially in less severe forms of THD, it often comes with side effects and tends to lose effectiveness over time. Effective disease-modifying therapies for THD are currently unavailable, making the development of alternative treatments a priority.

Recently, the interaction of TH with the cochaperone DNAJC12 has been reported. TH and DNAJC12 form a high-affinity complex which increases the stability of TH and delays its aggregation. We hypothesize that the loss of integrity and catalytic efficiency in THD patients might be compensated by its interaction with DNAJC12, and we propose the stabilization of the TH:DNAJC12 complex as an innovative target to develop potential treatments for THD. To achieve that, our research is focused on searching for pharmacological chaperones using high-throughput screening (HTS). The HTS performed with the Prestwick Chemical library® (1520 compounds, most FDA- and EMA approved) has revealed a promising compound (Hit 1) that binds both to TH and the TH:DNAJC12 complex, stabilizing it, and delaying TH aggregation, with minor effect on its catalytic activity.

The discovery of Hit 1 largely proofs the pharmacological chaperone concept and the value of stabilizing the TH:DNAJC12 complex as a new therapeutic strategy to develop potential treatments for THD and other dopamine deficiencies.

(10) Discovery and characterization of CRISPR-Cas endonucleases from cold-adapted bacteria

Greta Daae SandsdalenI[1], Maryam Imam[2], Ole Morten Seternes[3], Adele Williamson[4] & Hanna-Kirsti Schrøder Leiros[1]

[1]Biomolecular and Structural Chemistry, Department of Chemistry, UiT The Arctic University of Norway, [2]The Norwegian College of Fishery Science, UiT The Arctic University of Norway, [3]Department of Phamacy, UiT The Arctic University of Norway, [4]The University of Waikato, New Zealand

The CRISPR-Cas genome editing system has revolutionized molecular biology, providing an array of biotechnological tools for carrying out precision genome modification and regulation. One limitation of the system at present is that most available tools are developed from and optimized for mesophilic organisms, which limits their utility in cold living organisms. This paucity of available knowledge on psychrophilic CRISPR-Cas systems and affiliated genome editing tools is particularly problematic for researchers of cold-blooded eukaryotes, where mismatched thermal preferences of the CRISPR components hinder application efficiency.

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 main goal is to discover and develop one or more CRISPR-associated endonucleases for efficient and precise genome editing at low temperatures. A second aim is to gain insights on CRISPR-systems across cold-adapted bacteria through bioinformatics analysis.

Our findings reveal a low prevalence of CRISPR-Cas systems in cold-adapted bacteria, compared to mesophilic and thermophilic species, where only 17.7% of the analyzed genomes contained CRISPR operons. Further, five CRISPR endonucleases were selected for experimental characterization. Currently, one of them shows promise for genome editing applications in low-temperature conditions.

The identification and initial characterization of Cas endonucleases from cold-adapted bacteria mark a pivotal step towards establishing a CRISPR-Cas platform optimized for salmonids. This advancement could significantly impact genomic studies and biotechnological applications for cold-adapted organisms, aligning with the goals of enhancing salmon health and aquaculture sustainability.

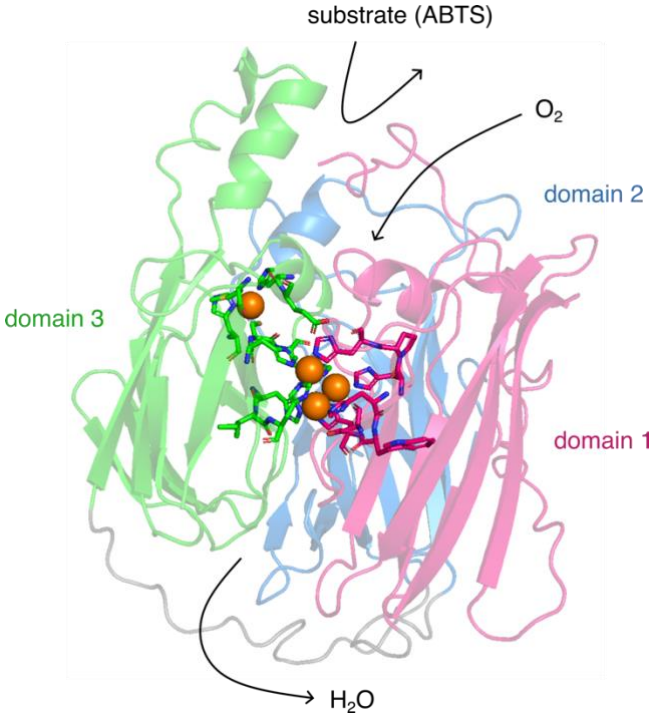
(12) Computational exploration of laccase cold adaptation

Helena Hradiská & Bjørn Olav Brandsdal

Hylleraas Centre for Quantum Molecular Sciences, Department of Chemistry, UiT The Arctic University of Norway, Tromsø, Norway

Laccase is a multicopper oxidase, which has found its use in many fields of industry and is also a promising biocatalyst in organic synthesis and bioremediation. To explore its possible application even further, one can search for homologs or engineer laccase to be efficient under extreme conditions such as low temperature. Even though the majority of produced laccase comes from fungi, this work focuses on bacterial laccases because they have been less studied in the past and cold-adapted bacterial homologs were recently found.

To model chemical reactions, one needs to run quantum mechanical (QM) calculations, however those are computationally demanding, especially for enzymes. Empirical valence bond (EVB) method allows us to run QM only for the reaction in solution and then use the calibration for the reaction in the enzyme and its homologs to determine reaction and activation Gibbs free energies. With EVB we can also obtain Arrhenius plots that inform about entropic and enthalpic contributions, thus about the temperature dependence of enzyme activity.



References:

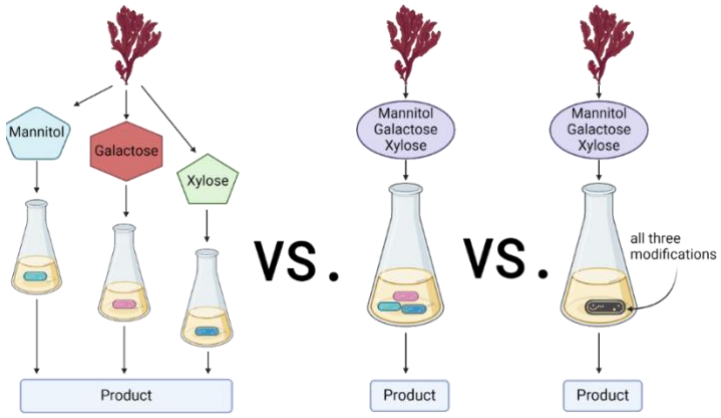
1. Åqvist, J., Isaksen, G. V.& Brandsdal, B. O. (2017). Computation of enzyme cold adaptation. Nature Reviews Chemistry, 1(7), 14, Article 0051. https://doi.org/10.1038/s41570-017-0051.
2. den Boer, D., de Heer, H. C., Buda, F.& Hetterscheid, D. G. H. (2023). Challenges in Elucidating the Free Energy Scheme of the Laccase Catalyzed Reduction of Oxygen. Chemcatchem, 15(1), 27. https://doi.org/10.1002/cctc.202200878.
3. Silva, C. S., Durao, P., Fillat, A., Lindley, P. F., Martins, L. O.& Bento, I. (2012). Crystal structure of the multicopper oxidase from the pathogenic bacterium *Campylobacter jejuni* CGUG11284: characterization of a metallo-oxidase. Metallomics, 4(1), 37-47. https://doi.org/10.1039/c1mt00156f.
4. Warshel, A.& Weiss, R. M. (1980). An empirical valence bond approach for comparing reactions in solutions and in enzymes. Journal of the American Chemical Society, 102(20), 6218-6226. https://doi.org/10.1021/ja00540a008.

(14) Co-cultivation of engineered *Corynebacterium glutamicum* strains for efficient use of seaweed-derived substrates

Ida Marie Stephansen[1] & Fernando Pérez-García[1*]

[1] Department of Biotechnology and Food Science, Norwegian University of Science and Technology, 7491 Trondheim, Norway
* Corresponding author: fernando.perez-garcia@ntnu.no

This project aims to meet the increasing demand for sustainable resources driven by a growing population. New renewable and alternative feedstocks are continuously sought in microbial biotechnology, and seaweed presents a promising solution. In particular, we aim to engineer the bacterium *Corynebacterium glutamicum*, which is commonly used as an industrial workhorse for the large-scale production of amino acids[1]. By applying molecular and synthetic biology tools, we will enable the utilization of seaweed-derived sugars like mannitol, xylose, or galactose by *C. glutamicum* strains[2]. Our approach will focus on co-cultivation, where each *C. glutamicum* strain will be engineered to utilize a specific sugar. We hypothesize that this method may improve the carbon conversion yield compared to classic monoculture approaches[3]. To support the concept of circular bioeconomy, utilization of seaweed sugars will be coupled with the production of added-value compounds like amino acids. Finally, to prove the potential of this idea, the newly established co-cultures will be scaled up in bioreactors using red and brown seaweed hydrolysates as the carbon source. Hence, this research seeks to contribute to sustainable production by tapping into the vast potential of ocean resources.



References:

1. Wolf, Sabrina, et al. "Advances in metabolic engineering of *Corynebacterium glutamicum* to produce high-value active ingredients for food, feed, human health, and well-being." *Essays in biochemistry* 65.2 (2021): 197-212.
2. Zhong, Haowei, et al. "The structural characteristics of seaweed polysaccharides and their application in gel drug delivery systems." *Marine drugs* 18.12 (2020): 658.
3. Pérez-García, Fernando, et al. "Dynamic co-cultivation process of *Corynebacterium glutamicum* strains for the fermentative production of riboflavin." *Fermentation* 7.1 (2021): 11.

Figure created with BioRender.com

(16) Enzyme display on *Bacillus subtilis* spores

Jan Benedict Spannenkrebs, Agnes Beenfeldt Petersen & Prof. Johannes Kabisch

NTNU, Institute for Biotechnology and Food Science

Bacillus subtilis can form metabolically dormant endospores in response to deteriorating environmental conditions such as poor nutrient availability. In a process called sporulation, a *Bacillus subtilis* cell divides asymmetrically. In one half of the cell a copy of the DNA is enclosed in multiple membrane and proteinaceous layers. Once sporulation concludes, the mother cell lyses, releasing the metabolically dormant spore into the environment. The formed spore provides protection for the DNA against extreme / harmful conditions, including high temperatures, desiccation, radiation and irritating chemicals. The spore core, which contains the DNA, is encased in an inner and outer coat. The outermost layer, designated the "crust," is primarily composed of six proteins (CotV, W, X, Y, Z, and CgeA)[1].

These crust proteins as well as others from different spore layers have already been used to create fusion proteins with proteins of interest, for example enzymes or antigens. For this, a copy of the fusion proteins DNA is inserted into *B*. *subtilis* under the control of a sporulation specific promoter. These fusion proteins self-assemble into the respective spore layer during sporulation. This system has for example been used for the display of a photodecarboxylase for the transformation of lipids to hydrocarbons[2], as well as for the display of the receptor binding domain of SARS-CoV-2[3]. The immobilization of proteins in this manner greatly facilitates downstream purification. Due to the spores' size, purification is possible by comparatively low-tech means like repeated centrifugation and washing.

A novel candidate class of enzymes for spore display are Alginate epimerases such as the processive AlgE-type. Alginates are linear polysaccharides comprised of linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G). The G-blocks can chelate divalent cations like Ca^{2+}, resulting in a hydrogel. The properties of the hydrogel can be modified by altering the ratio and sequence of the M and G blocks, making them an interesting product for example the food and pharmaceutical industry[4]. Alginate epimerases are enzymes capable of epimerizing M into G blocks, thus modifying the alginates properties.

References:

1. Bartels, J., Blüher, A., López Castellanos, S., Richter, M., Günther, M. and Mascher, T. (2019), The *Bacillus subtilis* endospore crust: protein interaction network, architecture and glycosylation state of a potential glycoprotein layer. Mol Microbiol, 112: 1576-1592.
2. Karava, M.; Gockel, P.; Kabisch, J. Bacillus Subtilis Spore Surface Display of Photodecarboxylase for the Transformation of Lipids to Hydrocarbons. *Sustainable Energy Fuels* **2021**, *5* (6), 1727–1733.
3. A. Vetráková, R. Kalianková Chovanová, R. Rechtoríková, D. Krajčíková, I. Barák, Bacillus subtilis spores displaying RBD domain of SARS-CoV-2 spike protein, Computational and Structural Biotechnology Journal, Volume 21, 2023, Pages 1550-making 1556.
4. Petersen AB, Tøndervik A, Gaardløs M, Ertesvåg H, Sletta H, Aachmann FL. Mannuronate C-5 Epimerases and Their Use in Alginate Modification. Essays in Biochemistry. 2023;67(3):615-27.

(18) Interactions of a quinoline-squaraine D-A-D triad with exfoliated graphene

Joseph Lumba, Maria Psarrou, Kristine Marie Halvorsen & Solon Economopoulos

Department of Chemistry, NTNU, Gløshaugen, 7030 Trondheim, Norway

Squaraine dyes have shown particular promise as a class of dyes exhibiting ultrastrong absorption properties that routinely cover a large part of the visible spectrum and even extend to the near-IR region.[1,2,3] On the other hand, the behavior of squaraine-based dyes in solution or thin films is subject to severe aggregation phenomena.[4] Therefore, detailed knowledge of the squaraine dye interactions in homogenic aggregates and with other species can be of importance for the design of new materials of desired properties.

In this study, a triad comprised of quinoline, squaraine and pyrene chromophores is synthesized and characterized. The resulting chromophore follows a Donor-Acceptor-Donor D-A-D architecture and exhibits very desirable optical characteristics for optoelectronic applications such as solar cells. The chromophores have been studied using steady state absorption and emission spectroscopy. To study the interaction of the light harvester with a well-known electron accepting material, exfoliated graphene nanoparticles are introduced in dilute solutions of the triad and the subsequent formation of H-type and J-aggregates is examined. Picosecond time-resolved fluorescence was used to try and probe the behavior of the observed interactions, while electrochemistry was used to rationalize the thermodynamically favorable pathways for energy or charge transfer in such complex nanoensembles.

(20) Discovery of a novel compound that stabilizes mitochondrial complex I - One step closer to the therapeutic intervention of Parkinson's disease

Kunwar Jung-KC[1,2], Svein I Støve[1,3] & Aurora Martinez[1,2]

[1]Department of Biomedicine, University of Bergen, Norway, [2]KG Jebsen Centre for Parkinson's Disease (DECODE-PD), University of Bergen, Norway, [3]Neuro-SysMed, Department of Neurology, Haukeland University Hospital, Norway

Parkinson's disease (PD) is a prevalent neurodegenerative movement disorder such as bradykinesia with resting tremor or rigidity, affecting approximately 2% of individuals aged 60 years and older. Mitochondrial dysfunction is strongly implicated in the aetiology of idiopathic and genetic PD. Since mitochondria are highly multifunctional organelles, their integrity is essential for neuronal function and survival. This study employs a cell-based screening approach to identify small molecule compounds capable of alleviating mitochondrial dysfunction in PD, specifically targeting complex I of the electron transport chain. Utilizing SHSY5Y cells as a model system, a systematic cell-based screening of Prestwick chemical library including 1520 FDA-approved drugs was conducted to identify compounds modulating complex I levels. The investigation identified a promising hit compound (Hit1), whose impact on mitochondrial level and function was assessed. Further assessment by SDS-PAGE indicated significantly increased levels of complex I in SHSY5Y cells upon treatment with Hit1. In addition, a significant enhancement in total oxidative phosphorylation (Oxphos) complexes in SHSY5Y cells was observed following treatment with Hit1. Also, complex I activity substantially increased, suggesting a potential therapeutic avenue for mitigating mitochondrial dysfunction in PD. The results indicate the potential of Hit1 as a drug-repurposing candidate for PD treatment, specifically addressing the mitochondrial dysfunction due to reduced complex I activity. This study also underscores the importance of leveraging cell-based screening techniques to identify novel compounds targeting specific disease pathophysiology, offering insights into potential avenues for therapeutic interventions. Overall, these findings contribute insights into the development of targeted interventions for PD, opening avenues for further preclinical and clinical investigations.

(22) From bacterial spores towards protein based, programmable biomatter

Magnus Philipp, Carina Dietz & Johannes Kabisch

Department of Biotechnology and Food Science, NTNU

Bacterial spores are among the most resilient biological systems in nature. In addition, they are biologically inactive, easy to handle and can be purified by simple centrifugation. These characteristics make spores an excellent target for biotechnology research and application. On an industrial scale, spores of the *Bacillus* genus are produced at several tons per year as seed additives to promote plant growth and act as pathogen antagonists. In research, the spore-surface-display technology has been developed that allows for the simultaneous production and immobilization of proteins which to date has mostly been applied for catalysis applications and the display of antigens and antibody formats. The display of the protein on the outside of the spore is achieved by genetic modification that introduces a synthetic gene encoding for a fusion of a spore surface protein and a protein of interest, controlled by a sporulation dependent promoter, resulting in protein display after sporulation.

In our current project we aim to expand the applications of *Bacillus subtilis* spores by developing them into a novel type of programmable biomaterial. To this end we display load-bearing proteins like e.g. spider silk, squid ring teeth protein and the *Bacillus subtilis* amyloid TasA on the surface of these spores. Load-bearing proteins are notoriously difficult to produce in heterologous systems due to their repetitive amino acid sequences and hydrophobic nature. However, displaying them on top of the spore negates problems such as inclusion body formation and allows the spores to serve as a seeding point for forming cheap biomaterials with novel properties. Once a baseline formula for a biomaterial has been developed, we aim to use existing knowledge such as the ability to use spores in 3D printing, hydrogels or concrete to further explore the properties of spore-based materials. In addition, we aim to further functionalize these materials by addition of upconverting nanoparticles and inorganic dyes to enable oxygen sensing and the use of light dependent enzymes.

(24) Characterization of the complex formed between phenylalanine hydroxylase and DNAJC12

Mary Dayne Sia Tai[1], Marte Innselset Flydal[2], Gloria Gamiz-Arco[1], Trond-André Kråkenes[1], Christer Flagtvedt Didriksen[1], Juha Pekka Kallio[1] & Aurora Martinez[1]

[1]Department of Biomedicine, University of Bergen, Bergen; [2]Department of Medical Genetics, Haukeland University Hospital

Hyperphenylalaninemia (HPA) primarily results from pathogenic variants in the PAH gene, which encodes phenylalanine hydroxylase (PAH), the enzyme responsible for converting L-Phe to L-Tyr. Variants of DNAJC12 also cause hyperphenylalaninemia along with dystonia, intellectual disability and neurotransmitter deficiencies in patients without any variants in PAH, in other tetrahydrobiopterin (BH4) dependent hydroxylases or in enzymes involved in BH4 synthesis or regeneration. As an Hsp40 protein, DNAJC12 binds to its client proteins, such as PAH, and presents them to Hsp70 for proper protein folding and homeostasis. However, the mechanism by which DNAJC12 binds to PAH is currently unknown. Human DNAJC12 and PAH, wild-type (WT) and HPA-associated variants, were recombinantly expressed in E. coli and purified before in vitro complex reconstitution. Biophysical and biochemical methods such as analytical size exclusion chromatography (SEC), native PAGE, immunoblotting and dynamic light scattering (DLS) were used to confirm complex formation and investigate the effect of complex formation on the stability of PAH. DNAJC12 and PAH form a complex that can be purified for further characterization. Results from SDS-PAGE, native PAGE and immunoblotting confirm the co-migration of DNAJC12 and PAH in non-denaturing conditions. By monitoring the time-dependent self-aggregation of PAH and HPA associated variants over time using DLS, DNAJC12 was also found to significantly delay PAH aggregation in vitro. Removal of an evolutionarily conserved octapeptide sequence in DNAJC12 was found to abolish its ability to bind to PAH, indicating the significance of this motif for DNAJC12 client binding. DNAJC12 recognizes and binds PAH through an evolutionarily conserved octapeptide sequence. The binding of DNAJC12 stabilizes PAH, preventing its self-aggregation over time.

(26) Selinexor potentiates BMP/SMAD-activity to induce apoptosis in multiple myeloma

Md Abu Hanif [1], Ingrid Quist-Løkken[2], Clara Andersson-Rusch[2,3] & Toril Holien[1,2,3,4]

[1]Department of Biomedical Laboratory Science, NTNU - Norwegian University of Science and Technology, Trondheim, Norway, [2]Department of Clinical and Molecular Medicine, NTNU - Norwegian University of Science and Technology, Trondheim, Norway, [3]Department of Hematology, St. Olav's University Hospital, Trondheim, Norway, [4]Department of Immunology and Transfusion Medicine, St. Olav's University Hospital, Trondheim, Norway

BACKGROUND: Multiple myeloma is a heterogenous cancer which requires new drugs to overcome relapse and resistance. Selinexor, an inhibitor of the XPO1 cargo protein, has been approved myeloma and cancers. Inhibiting XPO1 with Selinexor could block major tumor suppressor proteins and trap mRNAs inferring oncoproteins, leading to apoptosis/cell death. BMPs activate canonical SMADs, causing apoptosis and cell growth arrest in myeloma cells. SMADs are believed to depend on XPO1 for nuclear-cytoplasmic transport.

AIM: We hypothesize that combining Selinexor and BMPs could potentiate apoptosis in multiple myeloma by increasing the retention of SMAD proteins in the nucleus. Moreover, Selinexor has severe side effects which urges for identification of new biomarkers. This can be easily done by investigating association of Selinexor with BMP-SMAD activity.

METHODS: Dose response curves exhibiting cell viability were generated where myeloma cell lines were treated with Selinexor and Eltanexor followed by combination with BMP6, and Activin B. Flow cytometry was used with annexin V-FITC to identify apoptosis. Primary CD138+ myeloma cells were treated with Selinexor and BMP6 alone or combination of them. Additionally, BRE-LUC reporter assay and immunoblot was used to identify SMAD activity and protein expression respectively. We also plan to explore localization and abundance of SMADs using various microscopic techniques

RESULTS: Selinexor mediated inhibition of XPO1 reduced cell viability in myeloma cell lines where Eltanexor has a more potent effect. Combining Selinexor and BMP6 leads to synergistic cell death in INA6 cell lines in different assays. Selinexor also potentiates BMP6 in primary CD138+ myeloma cells. We plan to use different microcopy techniques to determine localization and abundance of SMAD proteins.

CONCLUSION: Our results indicate that Selinexor treatment cooperates with BMP-SMAD activity to induce apoptosis in multiple myeloma. It would decipher the mechanistic relevance of the synergistic cell death approach in multiple myeloma. This detailed mechanistic knowledge could help to identify biomarkers for Selinexor efficacy to predict patient response to it.

(28) Detailed characterization of hardwood and softwood lignin conversion by a brown-rot basidiomycete

Morten Rese[1], Gijs van Erven[2,3], Romy Veersma[3], Gry Alfredsen[4], Vincent G. H. Eijsink[1], Mirjam A. Kabel[3] & Tina R. Tuveng[1]

[1]Faculty of Chemistry, Biotechnology, and Food Science, Norwegian University of Life Sciences (NMBU), P.O. Box 5003, 1433 Ås, Norway, [2]Wageningen Food and Biobased Research, Bornse Weilanden 9, 6708 WG, Wageningen, The Netherlands, [3]Wageningen University & Research, Laboratory of Food Chemistry, Bornse Weilanden 9, 6708 WG, Wageningen, The Netherlands, [4]Norwegian Institute of Bioeconomy Research, Department of Wood Technology, P.O. Box 115, NO-1431, Ås, Norway

Wood-degrading brown-rot fungi primarily target carbohydrates, while lignin becomes partially modified and of potential interest for targeted lignin valorization[1]. Here, we report a comprehensive comparison of lignin conversion by a brown-rot basidiomycete, *Gloeophyllum trabeum*, growing on a hardwood and a softwood substrate. By harnessing the latest advancements in analytical methodologies, we show that *G. trabeum* not only degrades polysaccharides efficiently and selectively but may also remove more lignin from wood than previously reported. Structure-wise, brown-rotted lignin appeared substantially C_α-oxidized, O-demethylated, depleted in interunit linkages, and enriched in diagnostic substructures indicative of C_α-C_β, β-O and O-4 bond cleavages in the β-O-4 aryl ether linkage. These findings enhance our understanding of lignin conversion by brown-rot fungi, revealing previously unknown aspects of this process. Specifically, despite the well-documented differences in lignin structure between hardwood and softwood[2], *G. trabeum* attacks the same bonds in the lignin structures, resulting in similar chemical modifications regardless of the wood substrate. Furthermore, we show that *G. trabeum* enhances the antioxidant capacity of the lignin, and that the residual lignin can be separated into low- and high-molecular weight fractions with distinct properties. This highlights the biotechnological potential of brown-rot fungi for developing lignin-based antioxidant or resin products.

References:

1. D. J. Yelle, J. Ralph, F. Lu, and K. E. Hammel, "Evidence for cleavage of lignin by a brown rot basidiomycete," *Environmental Microbiology*, vol. 10, no. 7, pp. 1844-1849, Jul. 2008.
2. W. Boerjan, J. Ralph, and M. Baucher, "Lignin biosynthesis," *Annual Review of Plant Biology*, vol. 54, pp. 519-546, Jun. 2003.

(30) Molecular insights into the differential toxicity of cholera toxin and heat-labile enterotoxin

Natalia Mojica[1], Flore Kersten[1,2], Albert Serrano[3], Joel B. Heim[1], Gabriele Cordara[1], Ken Teter[3] & Ute Krengel[1]

[1]Department of Chemistry, University of Oslo, [2]Centre for Molecular Medicine Norway (NCMM), University of Oslo, [3]Burnett School of Biomedical Sciences, University of Central Florida

Cholera toxin (CT) and heat-labile enterotoxin (LT) are two similar AB_5 toxins responsible for the diarrhea characteristic of *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) infections. They consist of a catalytically active A1 subunit, an A2 linker, and a pentamer of cell-binding B-subunits [1]. Both toxins bind to the same GM1 surface receptor on the host cells and have similar levels of enzymatic activity, yet CT is more potent than LT, making cholera the more severe disease. The difference in toxicity has been attributed to structural differences near the C-terminus of the A2 linker (amino acid residues 226-236) [2], but the underlying molecular mechanism remains unknown. Recently, we showed that toxin disassembly by protein disulfide isomerase (PDI), which is a key event in the intoxication process, is more efficient for CT than for LT[3]. We hypothesized that the difference in toxin disassembly is related to the positioning of the A1 subunit relative to the B-pentamer[3] (Figure 1).

Here, we determined the crystal structures of two cholera toxin variants, where either one (D229E) or four (D229E, I230V, T232I, H233Y) amino acid residues in the critical A2 linker sequence were substituted for the residues present in LTA2 (Figure 1; colored residues within the pores of the grey pentamers). The results of this structural analysis will be presented here.

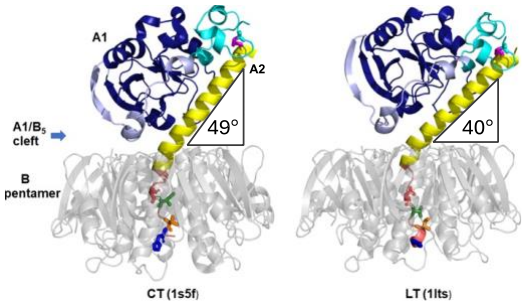


Figure 1. Structures of wild-type CT and LT [3]. For the two toxins, the angle of the A1 subunit relative to the B-pentamer differs by 9 degrees.

References:

1. Heggelund, J. E., Bjørnestad, V. A., and Krengel, U. (2015) *Vibrio cholerae* and *Escherichia coli* heat-labile enterotoxins and beyond, in *The Comprehensive Sourcebook of Bacterial Protein Toxins*, pp 195–229. Elsevier Ltd.
2. Rodighiero, C., Aman, A. T., Kenny, M. J., Moss, J., Lencer, W. I., and Hirst, T. R. (1999) Structural basis for the differential toxicity of cholera toxin and *Escherichia coli* heat-labile enterotoxin. *Journal of Biological Chemistry 274*, 3962–3969.
3. Serrano, A., Guyette, J. L., Heim, J. B., Taylor, M., Cherubin, P., Krengel, U., Teter, K., and Tatulian, S. A. (2022) Holotoxin disassembly by protein disulfide isomerase is less efficient for *Escherichia coli* heat-labile enterotoxin than cholera toxin. *Sci Rep 12*, 34.

(32) Biomass revalorization with engineered *Corynebacterium glutamicum* towards sustainable bioprocesses

Simen Jervell Lund[1], Fernando Pérez-García[1] & Trygve Brautaset[1*]

[1] Department of Biotechnology and Food Science, Norwegian University of Science and Technology, 7491 Trondheim, Norway
* Corresponding author: trygvebrautaset@ntnu.no

This project addresses the rising demand for sustainable resources due to population growth. In microbial biotechnology, there's a continuous search for renewable and alternative feedstocks in order to avoid food-competitive materials and fossil-based substrates (Perez-Garcia et al. 2022[1]). Our goal is to engineer the bacterium *Corynebacterium glutamicum*, a widely used industrial workhorse for large-scale amino acid production (Wolf et al. 2021[2]). Through the application of molecular and synthetic biology tools, we will enable the use of carbohydrate containing substrates such as spent grain from breweries, side streams from potato industry, and agricultural harvest residues. We will develop *C. glutamicum* strains for the degradation of the polymer starch into glucose, and for the utilization of the sugar pentoses xylose and arabinose as well as the sugar hexoses mannose and galactose (Wendisch et al. 2016[3]). To advance the concept of a circular bioeconomy, our approach will couple the utilization of carbohydrates with the production of high-value compounds such the amino acids and amino acids derivatives. To demonstrate the viability of this strategy, the newly developed microbial strains will be tested in lab-scale bioreactors using real hydrolysates from the aforementioned substrates as the carbon source. This research aims to contribute to sustainable biotechnology by integrating new and renewable biomass resources into biotechnological processes.

References:

1. Pérez-García, F., Brito, L. F., Irla, M., Jorge, J. M.& Sgobba, E. (2022). Promising and sustainable microbial feedstocks for biotechnological processes. Frontiers in Microbiology, 13, 973723.
2. Wolf, S., Becker, J., Tsuge, Y., Kawaguchi, H., Kondo, A., Marienhagen, J., ... & Wittmann, C. (2021). Advances in metabolic engineering of Corynebacterium glutamicum to produce high-value active ingredients for food, feed, human health, and well-being. Essays in biochemistry, 65(2), 197-212.
3. Wendisch, V. F., Brito, L. F., Lopez, M. G., Hennig, G., Pfeifenschneider, J., Sgobba, E.& Veldmann, K. H. (2016). The flexible feedstock concept in industrial biotechnology: metabolic engineering of Escherichia coli, Corynebacterium glutamicum, Pseudomonas, Bacillus and yeast strains for access to alternative carbon sources. Journal of Biotechnology, 234, 139-157.

(34) Exploring the dynamics of carbohydrate-binding modules using NMR

Susanne Hansen Troøyen, Davide Luciano & Gaston Courtade

Department of Biotechnology and Food Science, Norwegian University of Science and Technology (NTNU). Trondheim, Norway

Several carbohydrate-active enzymes contain carbohydrate-binding modules (CBMs) that regulate enzymatic activity by localizing the catalytic domain towards the surface of insoluble substrates such as cellulose [1]. Recently, some CBMs have also been shown to have affinity for non-natural substrates such as polyethylene terephthalate (PET) [2, 3]. CBMs are thought to act as anchors on the substrate surface, allowing the enzyme to perform its activity inside a radius limited by the length of the flexible linker connecting the two domains. However, as illustrated by the Sabatier principle, the CBM should not stay attached at the same position too long – otherwise it would limit the catalytic efficiency of the enzyme. The dynamics of exchange between the free and bound state of the binding module is thus an important, but largely unexplored property of these proteins. NMR spectroscopy offers an opportunity to study protein exchange processes through carefully chosen experiments such as dark state exchange saturation transfer (DEST), solvent paramagnetic relaxation enhancement (sPRE) and relaxation rate measurements. We present here our ongoing investigation into CBM binding dynamics and identification of their substrate-binding site. In combination with affinity assays and kinetic experiments, we anticipate that these insights will contribute to developing our understanding of CBM binding mechanisms.

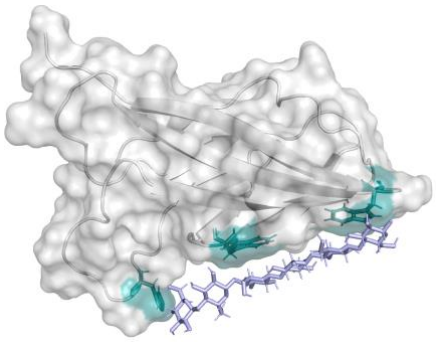


Figure 1. CBM2 from *Streptomyces coelicolor* with cellohexaose ligand.

References:

1. Courtade, G., Forsberg, Z., Heggset, E. B., et al. (2018) The carbohydrate-binding module and linker of a modular lytic polysaccharide monooxygenase promote localized cellulose oxidation. *J Biol Chem* 293(34), 13006 –13015.
2. Weber, J., Petrović, D., Strodel, B. et al. (2019) Interaction of carbohydrate-binding modules with poly(ethylene terephthalate). *Appl Microbiol Biotechnol* 103, 4801–4812.
3. Rennison, A. P., Westh, P., and Møller, M. S. (2023) Protein-plastic interactions: The driving forces behind the high affinity of a carbohydrate-binding module for polyethylene terephthalate. *Sci Total Environ* 870, 161948.

(36) Transcription factor network in osteosarcoma

Xuan Thang Nguyen[1,2], Emily Martiensen[1,2], Marie Rogne[1,2], Bernd Thiede[3] & Ragnhild Eskeland[1,2]

[1]Institute of Basic Medical Sciences, Medical Faculty, University of Oslo, Norway, [2]Centre for Cancer Cell Reprogramming (CanCell), Centre of Excellence, [3]Department of Biosciences, University of Oslo, Norway.

Transcription factors, as vital regulatory proteins, bind to DNA, orchestrating the activation or deactivation of genes and governing the transcription of messenger RNA (mRNA) crucial for protein synthesis in all cells and organisms. Mutated or dysregulated transcription factors can significantly impact cell growth, dominated by the protein network, and can lead to cancer progression. Our study, which investigates the network of transcription factor regulation in osteosarcoma, has the potential to emphasize the need for further investigation significantly. We used a combination of proteomics and transcriptomics analysis to map transcription factor networks in three different aggressive of human osteosarcoma cell lines. Our findings on master regulators and pioneer transcription factors associated with higher aggressiveness in osteosarcoma, including the RUNX, HGM, and FOX family's transcription factors, could inspire new directions for drug screening and potential treatment strategies.

(38) Simulating antimicrobial peptide interactions with lipid bilayers

Tonje Reinholdt Haugen[1], Laura Erntsen[1] & Bjørn Olav Brandsdal[1]

[1]Hylleraas Centre for Quantum Molecular Sciences, Department of Chemistry, UiT The Arctic University of Norway, Tromsø, Norway

Antimicrobial peptides (AMP) are a ubiquitous part of the host defense systems of many organisms, ranging from fungi, and bacteria to mammals. The have proven to have a wide range of use, showing not only antimicrobial properties, but also showing antiviral and antifungal properties. AMPs can be classified as oligopeptides with less than 100 amino acids and a cationic charge and they were first reported in 1939, the same year Florey and Chain started their work purifying penicillin. In this study, 120 different cyclic hexapeptides were screened and the top 4 were further analyzed using both computational and experimental methods. This poster will focus on the computational part of the analysis.

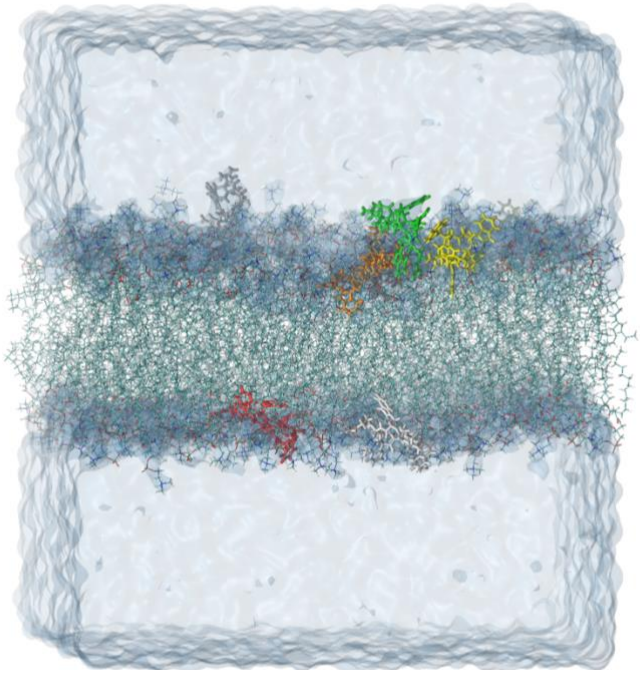


Figure 1. Showing one of the systems modelled with the cyclic peptides (shown in different different colours) and a lipid bilayer.

The computational analysis has done by using molecular dynamics at atomistic level with the 4 different peptides in water and in combination with two models of the inner membrane of *E. coli*. To prevent the simulations to be too computationally demanding, a small patch of about 100-150 lipids per lipid layer was used in the simulations. The two bilayer models used in the computational analysis were a POPE and POPG lipid bilayer with 3:1 ration and a DMPC and DMPG lipid bilayer with a 1:20 ratio. The second model was constructed as a more direct comparison with experimental results. Comparing the models with and without the lipid bilayer, also lets us investigate what conformational differences there may be in the peptides depending on environment

References:

1. Bahar, A. A.; Ren, D. Antimicrobial Peptides. *Pharmaceuticals* **2013**, *6* (12), 1543–1575. https://doi.org/10.3390/ph6121543.
2. Hong, C.; Tieleman, D. P.; Wang, Y. Microsecond Molecular Dynamics Simulations of Lipid Mixing. *Langmuir* **2014**, *30* (40), 11993–12001. https://doi.org/10.1021/la502363b.
3. Sarre, B. R. MD Simulations Reveal How Synthetic Antimicrobial Peptides Interact with Membrane Models. **2014**, No. December.

(40) Unleashing the potential of therapeutics: The promise of monodisperse PEGs

Yomkippur Perez[1], Erik Agner[2] & Magne Olav Sydnes[3]

[1]University of Stavanger, [2]Polypure, [3]University of Bergen

Polyethylene glycol (PEG) stands as a premier biocompatible polymer extensively employed in medical applications. Its usage spans across a wide spectrum of pharmaceutical products, including oral drugs, topical medications, and most prominently, in the COVID-19 vaccines. Industrially produced PEG typically exhibits polydispersity, consisting of single PEG chains with at least 10 different lengths. Conversely, monodisperse (uniform) PEGs comprise a single PEG chain length, albeit they are less common and available in smaller quantities at a higher cost.

In this study, we pioneered methods for producing monodisperse PEG derivatives essential to the pharmaceutical industry. Specifically, we established pathways for synthesizing high molecular weight monodisperse PEGs over 1000 Da. We also developed synthetic routes and purification methods for in-demand monodisperse PEG-lipids, such as DMG-PEG 2000, utilized in Moderna's COVID-19 vaccine, Spikevax®. Additionally, we created a diverse repository of PEG-peptides, evaluating them as potential candidates for pancreatic cancer vaccines.

(42) Structural analysis of chorismate mutase variants

Luca Streitlein, Mateu Montserrat Canals and Ute Krengel

Universitetet i Oslo, Department of Chemistry

Chorismate mutase is a key enzyme in the shikimate pathway, where it catalyses the pericyclic Claisen rearrangement of chorismate to prephenate. It plays a central regulatory role in the pathway by directing the synthesis towards phenylalanine and tyrosine instead of tryptophane. As the shikimate pathway is absent in mammals, better understanding of the structure and function of chorismate mutase could lead to its exploitation as a target for new antibiotics, fungicides and herbicides.

The focus of this study lies on the characterization of the chorismate mutase from *Corynebacterium glutamicum*, which has limited activity on its own and requires activation by a partner enzyme, D-arabino-heptulosonate-7-phosphate synthase. Our collaborators at the ETH, led Dr. Peter Kast (ETH), have recently created enzyme variants of chorismate mutase with directed evolution that are highly active on their own. Structural characterization will be performed by single crystal X-ray diffraction and comparison of the conformations adopted by the enzymes, to deepen our understanding of the activity and regulation of chorismate mutase.