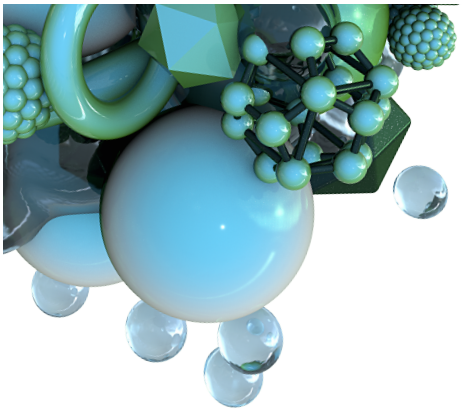

Abstract Book

Digital Life Conference 2019

CENTRE FOR DIGITAL LIFE NORWAY

Content

Program

Day 1, Thursday 5. September

10:00 Registration and poster setup

11:00 Welcome – Trygve Brautaset, Centre Leader

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Strategic priorities within digitalisation and its implications for research and innovation

11:30 **Opening keynote - John Sigurd M. Svendsen**

On the science of tackling antimicrobial resistance

13:00 Lunch

14:00 **Keynote - Jennie Ekbeck**

On the innovation journey within biotech research

15:00 **Keynote - Heidrun Åm**

Digital Life Norway and value creation: Which Common Good Do We Talk About?

15:30 Coffee break

16:00 **Poster sessions**

16:00-17:15 Presentation of odd numbered posters

17:15-18:30 Presentation of even numbered posters

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Day 2, Friday 6. September

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One citizen – one digital twin and the future AI-based health ecosystem for self-management (wearables) and decision-support (medical imaging)

09:45 **Short selected talks**

09:45 Mohammed Sunoqrot, NTNU: *A quality control system for automated prostate segmentation on T2-weighted MRI*

10:00	Daria Zaytseva-Zotova, NTNU: *Alginate hydrogels with tailored mechanical and biological properties for 3D fibroblast culture*
10:15	John Zobolas, NTNU: *DrugLogics: Ensemble model analysis unravels drug synergy mechanisms*
10:30	Eskil André Karlsen, UiT: *Developing a new generation of catheters*
10:45	Coffee break
11:15	**Keynote - Kjetill S. Jakobsen**
	Biodiversity genomics: Sequencing all life on earth for the benefit of mankind
12:00	**Closing Keynote – Ursula Klingmüller**
	TBA
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14:00	Ask a coordinator / Project meetings

Keynote speakers

Nikolai Astrup

Minister of Digitalisation

The Minister of Digitalisation is responsible for ICT policy in the Ministry of Local Government and Modernisation. The Minister is also responsible for the work on electronic communications, including responsibility for the Norwegian Communications Authority. In addition, the Minister is responsible for the Altinn portal, business-oriented ICT, the Digital21 strategy for digitalisation of businesses in Norway, and resources for ICT research.

John S. Mjøen Svendsen

Professor of organic chemistry, UiT – The Arctic University of Norway, Chief Scientific Officer, Amicoat AS, and Project Leader, DigiBiotics

John Sigurd Mjøen Svendsen is the key researcher behind several of the large research projects at UiT where the common denominator is the quest to find molecules with features that can be used effectively in pharmaceuticals. The search area is – conveniently for the Tromsø researchers – where mollusks, marine fungus and micro algae gather: the ocean.

Material from the ocean and the ocean bed has been sampled by researchers for over a decade. Among other pharmaceuticals, John Sigurd and his colleagues are investigating whether the collected samples contain molecules that potentially can be developed into the new Antibiotics. Going through the samples in search of new drugs is not new. What is new with the approach, is that the researchers are developing new methods that enable improved analysis of the samples and molecules, than what was possible prior.

– We want to search for new Antibiotics where no one has searched before; we search for larger molecules with antibiotic features, larger than the small ones that were discovered many years ago, John Sigurd said to a local Tromsø paper earlier this year.

An important factor that enables this approach; to develop new methods, is the super computer UiT inaugurated two years ago. The computer has the largest computational capacity in Norway, larger than all other current computational systems in the country – altogether. John Sigurd's research projects are a heavy client of the super computer.

The unique computer is also a vital tool in another of John Sigurd's projects, where they are searching for marine molecules to use in pharmaceuticals to combat an enzyme believed to cause Alzheimer. – Once we have the three best candidates of this molecule, my work with this is over. Then remains the testing on animals and patients, and then preparations for manufacturing. We are going to move from mg to kg, so we need to build industry, said John Sigurd in this interesting article in Teknisk Ukeblad: Skal kurere Alzheimer med norskutviklet medisin (in Norwegian).

On the science of tackling antimicrobial resistance

Let there be no doubt, the antimicrobial resistance crisis is real! Even the fact that Norway has been sheltered against the many of the most resistant pathogens, it is still a fact that the

number of MRSA carriers in Norway have more than tripled in the period between 2010 and 2015, and the number of community-acquired and imported MRSA cases have doubled in the same period. However, in a global perspective, the rise in antimicrobial resistance is already a full-fledged crisis, and it has been projected that by 2050, more people will die of infection than of cancer, and that the cumulative global cost in lost production will have amounted to 100 trillion USD. The reasons for the growing resistance crisis is manifold, and there is certainly no quick fix to the problem. Governments and NGOs around the world has devised plans to combat the emerging resistance crisis, and the Norwegian government has decided that Norway should be a driver in international and normative work to improve access, responsible use, and development of new antibiotics, vaccines and better diagnostic tools. Digital Life Norway (DLN) hosts several projects that aims to fulfill some of these goals, and UiT, The Arctic University of Norway has consolidated and strengthened its effort by creating a Centre of new antimicrobial strategies, CANS.

Digibiotics, a DLN project, and an important part of CANS, has as its goal to translate innovative scientific discoveries into commercially attractive propositions by combining in-depth scientific knowledge with a broad understanding of the requirements of successful drug discovery and development. DigiBiotics is

- Digitally mining genomes, isolating and identifying novel antimicrobial molecules from Arctic marine microorganisms with focus on compounds less prone to trigger resistance development
- Creating, developing and validating experimental and computational methods for determination of molecular structure, absolute configuration and conformation
- Provide, through new computational and experimental methods, an in-depth understanding of structure-activity relationships for “middle-space molecules” enabling optimization of their biological activity
- Compute quantitative structure activity relationships (QSAR) for knowledge-driven refinement of antimicrobial molecules into compounds with acceptable pharmacokinetics, optimizing potency, absorption, distribution and metabolic properties
- Define the microbial targets and resistance development to provide feedback for refined molecular design

The lecture aims to give a scientific background for the antimicrobial resistance crisis and how DLN through projects like DigiBiotics will participate and contribute to the global effort to stem the tide of antimicrobial resistance.

Jenny Ekbeck

CEO Umeå Biotech Incubator, Sweden

Jennie Ekbeck has the rare honor of being decorated as «Innovations Angel» – an award given to a distinct person who contributes to a better innovation climate in Sweden. Ekbeck earned the title through her work at the Umeå Biotech Incubator (UBI), by improving the possibilities for Swedish scientists to approach the business world, and creating commercial products from ideas arising from their research.

At UBI we work with people who are very competent and experienced within their field, but when they come to us they are novices… We match them with people who are as experienced

as them, in drug- or product development, an equal partner with complementary know-how, she says in one interview.

Ekbeck is an expert on how to develop research within the Life Sciences into commercial businesses and has been invited speaker at Future Sweden Danish Life Science and Life Science Days, inspirational speaker at Swelife and contributed to panel conversations at Sweden bio and the Swedish Foundation for Strategis Research. She has experience with lecturing for both scientitsts and students.

Heidrun Åm

Senior Researcher at the Centre for Technology and Society at the Department for Interdisciplinary Studies of Culture, NTNU

Over multiple years, Heidrun Åm has explored the governance of science and technology in her research, emphasizing mainly on nano- and biotechnology, sustainable energy and society, and interdisciplinarity. Her political science background is an important contribution to the society-oriented mission of the Centre for Digital Life Norway. Consequently, it is perhaps no surprise that Heidrun also leads a DLN-project exploring questions of social responsibility and social concerns arising from computational biotechnology: «Res Publica. Responsibility, practice, and the public good across Digital Life».

Digital Life Norway and value creation: Which Common Good Do We Talk About?

The mandate of the Centre for Digital Life Norway is to create economical, societal, and environmental value in Norway. In pursuit of value creation, economic goods and marketization are often important themes. However, the question of the public or common good may become backgrounded. In this presentation, I want to put the common good center stage and map which common good we talk about when we talk about value creation in DLN. In this mapping analysis, I pursue a framework and research program recently published by Tamar Sharon (2018). My aim is to make explicit the different approaches towards the common good identified in DLN's work and research: a market-oriented common good (economic growth), an industrial-oriented common good (increased efficiency), a project-oriented common good (network building), a fame oriented common (recognition), a vitality-oriented common good (greater health), and a civic-oriented common good (collective well-being). These different attitudes to the goal or common good in DLN influence daily decision-making. Therefore, it is important to elicit such orientations to critically evaluate them for their internal consistencies and against each other. I will argue that DLN needs to decide what shall be the most legitimate conception of common good in DLN and position itself against the drawbacks of simultaneously pursuing too many goals.

Sharon, T. (2018). When digital health meets digital capitalism, how many common goods are at stake? Big Data & Society.

Frank Lindseth

Professor at Department of Computer and Information Science (NTNU) and Senior Research Scientist at SINTEF Medical Technology.

Frank Lindseth is a professor in the computer science department at NTNU and a core member of the Norwegian Open AI Lab. His research field is visual computing with special focus on computer vision, an area completely taken over by data-driven methods and AI. Lindseth has over 20 years of experience in the medical field, working at SINTEF Medical Technology and the national advisory unit for ultrasound and image-guided therapy in close collaboration with surgeons at St. Olav university hospital. Furthermore, Lindseth has been the project leader of several medical image computing related projects and the application of computer vision and AI methods in the medical field is still one of his key interests.

One citizen – one digital twin and the future AI-based health ecosystem for self-management (wearables) and decision-support (medical imaging)

The Norwegian healthcare system is among the best, and it's available for everybody. On the other hand, the healthcare system of today will not be sustainable in the future and there are key issues related to the citizens and their health data, as well as how we generate and use new knowledge extracted from these data. The presentation will address these issues and show how the digital twin concept and its associated ecosystem can mitigate some of these challenges in the age of IoT, Big Data, AI and cloud computing.

Kjetill S Jakobsen

Professor at Centre for Ecological and Evolutionary Synthesis (UiO)

The research of Kjetill S. Jakobsen has dealt with quite diverse aspects of evolutionary genetics and genomics. A main theme in his work relates to the genetic forces (both ongoing and historical) acting upon populations, and how the genetics relates to ecological and evolutionary processes. In this context, issues such as phenotypic plasticity, selective forces and epigenetic phenomena are of special interest.

Essential ingredients in his interdisciplinary approach are genomics, bioinformatics, statistics and phylogeny, combined with evolutionary and ecological thinking. Through this, he works on bridging the gap between organism-oriented research (evolutionary and ecological research), and genomics/molecular biology (phenotype-genotype issues).

At Centre for Digital Life Norway he is the workgroup leader of outreach and in-reach communication and is valued by his colleagues for his vast experience, enthusiasm and ability to be outspoken in a very constructive manner.

Biodiversity genomics: sequencing all life on earth for the benefit of mankind

Over the last decade sequencing technologies have undergone a tremendous development. Recently, long-read sequencing in combination with other long-range techniques has made it possible to generate chromosome-level assemblies at a relatively modest cost. This

development has boosted a generation of high-quality reference genomes for both model and non-model organisms. Furthermore, it has catalyzed the birth of large sequencing efforts such as the Earth Biogenome Project (EBP). The EBP has been termed «a moonshot for biology» aiming at sequencing all eukaryotic species on earth within a ten-year period. The overarching goals are to protect biodiversity, under threat by climate change and anthropogenic activities, to better understand ecosystems, and benefiting human welfare (new treatments of various disease, drug development, synthetic fuels, new biomaterials, new or improved food production). Thus, EBP aims at developing and supporting a sustainable green bio-economy. Norwegian universities and research institutions have joined EBP through a «Memorandum of Understanding». There are several new developments and ongoing activities regarding chromosome level assemblies initiated by Norwegian research environments. These new developments and future possibilities and perspectives of biodiversity genomics will be discussed.

Ursula Klingmüller

Professor (Dr.) and head of the research division Systems Biology of Signal Transduction at the German Cancer Research Centre (DKFZ) in Heidelberg.

Ursula Klingmüller is trained in Cell Biology and Molecular Virology from Heidelberg University, Harvard Medical School in Boston and Whitehead Institute for Biomedical Research, Cambridge (US). She is since 2016 an elected member of the German Ethics Council and serves in several editorial boards for scientific journals, e.g. since 2015, for Nature Systems Biology and Applications.

The Klingmüller group uses a combination of experimental and theoretical approaches to understand the complex interplay between cell behaviour and signal transduction processes that drive cancer development and tissue generation. The mathematical models generated are used to identify pathological mechanisms and possible intervention strategies. Using this interdisciplinary approach, the research group has contributed with experimental-based mathematical models of key signalling pathways involved in hematopoietic development and malignancies, liver regeneration and lung cancer, to mention some.

The interdisciplinary approach taken is further developed towards application in systems medicine and personalized medicine. Here, they use their developed mathematical models and adapt them to different patient groups or even individual patients for use as tools to optimize treatment protocols.

Selected short talks

A quality control system for automated prostate segmentation on T2-weighted MRI

Author: Mohammed Sunoqrot, NTNU

Introduction:
Computer-aided detection and diagnosis (CAD) systems have been proposed to overcome the limitations of radiological reading of multiparametric MRI[1]. A primary step in an efficient prostate CAD system is fully automated segmentation of the prostate[2]. The performance of the most promising automated prostate segmentation methods, in most cases deep learning-based (DL) methods, is approximately equal to that of comparing segmentations of one expert with another[3]. However, manual quality control (QC) is still necessary, as poorly segmented prostates may lead to poor CAD performance and inaccurate diagnosis. Our aim was therefore to establish a fully automated QC system for prostate segmentation based on T2-weighted MRI.

Methods:
An overview of the proposed method is shown in Figure 1.
Using a deep learning-based segmentation method[4], the prostate gland was segmented for 404 patients collected from publically available datasets[5-7]. The dice similarity coefficient (DSC) was calculated to evaluate the segmentation accuracy in comparison to expert delineations. The interquartiles Q1, Q2 (median) and Q3 of the DSC distributions and the Lower Tukey Fence (LTF), defined as Q1 – 1.5*(Q3-Q1), were calculated. The segmentations were categorized into 4 groups: Group 1 (poor, DCS ≤ LTF), Group 2 (lower intermediate, LTF < DSC ≤ Q1), Group 3 (upper intermediate, Q1< DSC ≤ Q2) and Group 4 (good, DSC > Q2). Then, series of features (Table 1, column 1 - 5) were extracted using four different scaling approaches (Table 1, column 6) from four areas of the segmented prostate masks (Table 1, column 7) to create multiple feature combinations. A decision tree bagger classifier[8] was trained and optimized on a randomly assigned training dataset (N=303) to find the feature combinations that best detected the poor segmentations, and subsequently validated on an independent validation dataset (N=101). This process was repeated 10 times to investigate the stability of the method.

Results and discussion:
Figure 2 presents the QC system's performance over the 10 models. These results indicate that the system was capable of detecting most of the poorly segmented cases, which is its main task, at the cost of erroneously flagging some of the intermediate and good cases as poor. In clinical practice, this could mean that a radiologist would avoid manually checking up to approximately 70% of the automated segmentations. Although slightly different feature combinations were selected in each of the 10 models, the validation performance was relatively stable. We also observed that the texture and shape features played an important role in all of the models. This is in the accordance with the way the DL algorithms work, gradually moving from shape-based to texture-based features through layers. A combination of extracted features from apex and middle of the prostate with a scaling approach would be preferred over others.

References:
1. Litjens G, et al. Computer-aided detection of prostate cancer in MRI. IEEE Trans Med Imaging 2014; 33(5): 1083-92.
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3. Shahedi M, et al. Spatially varying accuracy and reproducibility of prostate segmentation in magnetic resonance images using manual and semiautomated methods. Med Phys 2014; 41(11).
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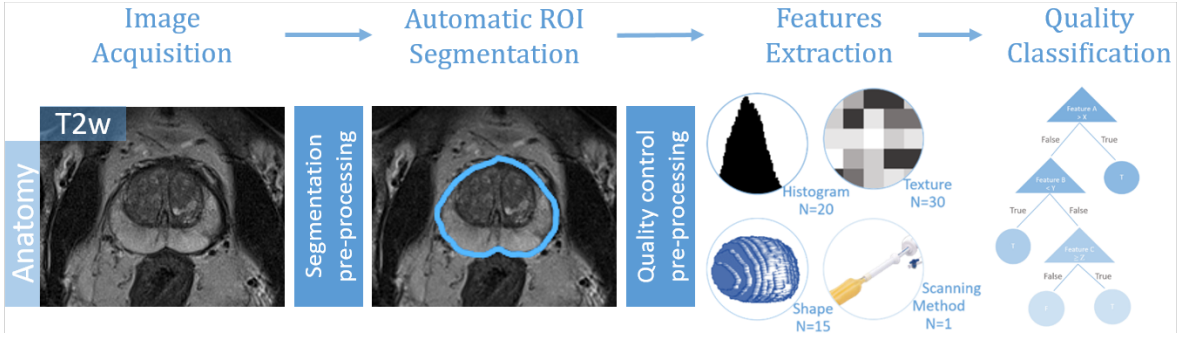


Figure 1. The pipeline of the proposed quality control system.

Figure 2. The classification errors as a measure of performance of the system during the training and validation over 10 models.

Table 1. List of the extracted features.

Histogram intensity features (N=20)	***Shape features (N=15)***	***Texture features * (N=30)***		***Scanning method feature (N=1)***	***Scaling approaches***	***The extraction area***
		GLCM (N=19)	***GLRLM (N=11)***			
10th percentile	Compactness 1	Autocorrelation	Gray-Level Nonuniformity	Has endorectal coil been used ?	Over the median of the middle slice intensity (1 slice) of the whole prostate	Whole prostate 3D volume
25th percentile	Compactness 2	Cluster Prominence	High Gray-Level Run Emphasis			
75th percentile	Elongation	Cluster Shade	Long Run Emphasis			
90th percentile	Equivalent Diameter	Contrast	Long Run High Gray-Level Emphasis		Over the median of the middle part intensity (multiple slices) of the whole prostate	Prostate apex
Coefficient of variation	Extent	Correlation	Long Run Low Gray-Level Emphasis			Middle of the prostate
Energy	Flatness	Difference entropy	Low Gray-Level Run Emphasis			
Entropy	Least Axis	Difference variance	Run Length Nonuniformity		Over the median of the whole prostate intensity	Prostate base
Interquartile Range	Major Axis	Dissimilarity	Run Percentage			
Kurtosis	Minor Axis	Energy	Short Run Emphasis			
Maximum	Solidity	Entropy	Short Run High Gray-Level Emphasis		No scaling applied	
Mean	Spherical Disproportion	Homogeneity	Short Run Low Gray-Level Emphasis			
Mean absolute deviation	Sphericity	Information measure of correlation 1				
Median	Surface Area	Informaiton measure of correlation 2				
Minimum	Surface Area to Volume ratio	Inverse difference				
Range	Volume	Maximum probability				
Root mean sruare		Sum average				
Skewness		Sum entropy				
Standard deviation		Sum of sqaures: Variance				
Uniformity		Sum variance				
Variance						

* Mean of angles ($\theta = 0^o, 45^o, 90^o$ and 135^o)

Alginate hydrogels with tailored mechanical and biological properties for 3D fibroblast culture

Author: Daria Zaytseva-Zotova, NTNU

Introduction

Cells of the human body normally grow in a three-dimensional (3D) environment surrounded by extracellular matrix (ECM). The ECM is essential for normal cell functioning: if cells lack 3D ordering and networking they can lose their tissue-like characteristics and phenotype. Alginate is an attractive polymer for fabrication of hydrogels that mimic native ECM. Alginate hydrogels have been shown to have in general good biocompatibility, tunable physical and mechanical properties, and they can be fabricated under physiological conditions allowing gentle entrapment of living cells. But alginates do not contain any specific binding sites for cell attachment.

The aim of our work is to develop hydrogels with mechanical and biological properties tailored for the specific needs of a given cell type or cell system, and in this case fibroblasts.

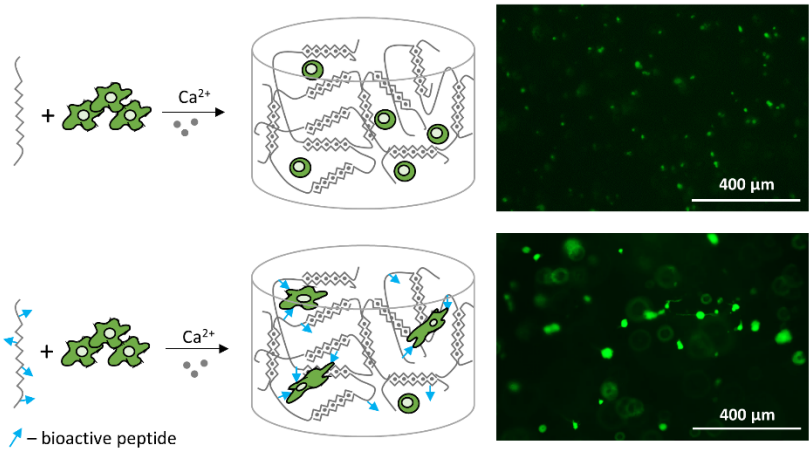
Methods

Bioactive peptides (IKVAV, YIGSR and GRGDSP), that mimic cell binding motifs of laminin and fibronectin, were grafted to alginates (F_G 0.68, M_W 240-300 kDa) using previously described method (Dalheim et al 2016). Hydrogels were crosslinked with calcium ions using internal gelation method – a well-known strategy for formation of soft gels with homogenous and reproducible structure (Draget et al 1990). Gelation kinetics and mechanical properties were characterized using oscillatory rheometry. For cell studies, hydrogels were casted in 96-well plates according to the protocol developed by us for robotic handling. Human fibroblasts were cultured on or within the hydrogels for up to 10 days. Every day cells were labeled using Live/Dead stain and examined under fluorescence microscope.

Result and discussion

In this work we created a library of alginate hydrogels having mechanical properties within the range typical for soft tissues (G' 10-1500 Pa) and functionalized with three model bioactive peptides. Gel formation procedure was optimized with regards to desired gelation kinetics for compatibility with the robotic system for future high-throughput screening of cell responses. Our preliminary results showed that the viability and morphology of fibroblasts depended on both mechanical and biological properties of the developed hydrogels, e.g. human lung fibroblasts (IMR90) favored softer gels functionalized with GRGDSP sequences (see graphical abstract).

This work is a part of 3DLife project within Center of Digital Life Norway, and is financed by the Research Council of Norway (project 269273).


Ca2+
400 µm
Ca2+
400 µm
– bioactive peptide

DrugLogics: Ensemble model analysis unravels drug synergy mechanisms

Author: John Zobolas, NTNU

Introduction

We have designed and developed a fully automated computational pipeline for drug response simulation and prediction in preclinical, cancer cell line model systems. This pipeline is able to produce cellular signaling network models that represent key cancer driving processes, actionable targets within these networks, and phenotypic output nodes (growing or dying) that provide an assessment of model performance when compared to actual observations. The logical modeling framework deploys a genetic algorithm that trains an *ensemble* of models to fit the steady state activity profile of a specific cancer cell line, as measured with proteomic, genomic and transcriptomic data. The platform represents our progress in the direction of providing predictions of pre-clinical efficacy, and ultimately clinical decision support for personalized medicine.

One of the goals of our research is to use computational modeling to understand why specific drug combinations work better (more synergistically) than others and identify possible regulatory network nodes that may serve as biomarkers that explain the *mechanism* by which these synergies happen.

Methods

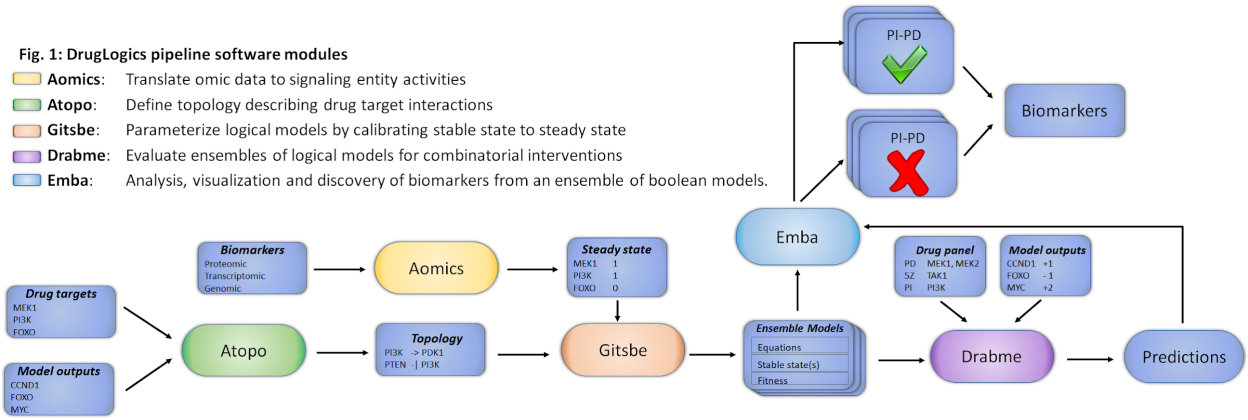
To investigate the mechanistic basis as represented in the computer models and identify the main model determinants influencing drug response synergy, we devised methods to split, analyze and compare the models for their predictive value of one or more experimentally observed synergies. We next arranged all the relevant computational methods to an R package and documented them for the ease of analysis and re-use by others, as well as for reproducibility purposes. We also plan to use several Machine Learning methods such as Linear Regression Models with Regularization (LASSO, Elastic Nets) and Random Forests (and effective combinations of them), to aid in the discovery of 'high leverage' network components that may serve as cancer biomarkers by extracting important features from the model ensemble dataset. These findings will be used to augment more signaling mechanism-based discoveries.

Results and discussion

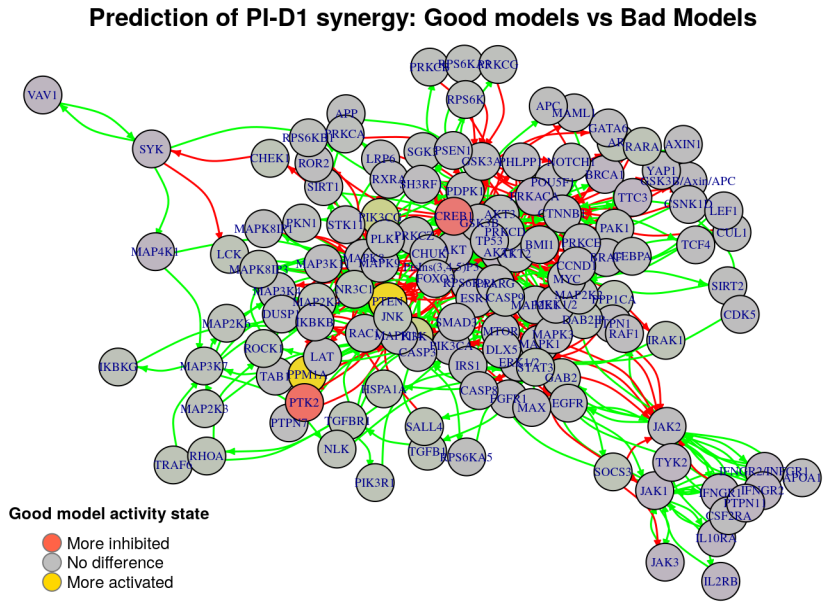
The results from our computational analysis deployed on the in silico representations of cancer cells and how these 'patient companion models' respond to drug perturbations in an effort to find biomarkers to assess drug response in cancer cell lines, can help us explain the mechanisms that favor or hinder the effective drug synergies and can ultimately be used to propose new combinations of drug targets that can be validated in wet-lab experiments. These experiments will either confirm our findings, helping thus build a more comprehensive knowledge map of cancer, or show us the need for improving our modeling efforts, practices and methodologies in order to better capture and explain the new model-driven, experimentally derived biomarkers.

Figures

The DrugLogics Pipeline:



A visual ensemble model drug prediction classification example:



Developing a new generation of catheters

Author: Eskil André Karlsen, UiT

Introduction

Central line-associated bloodstream infections are a major concern for the healthcare system. According to the Central for Disease Control and Prevention, approximately 41000 incidents of bloodstream infections happen annually that is associated with a central line catheter, and it is estimated that up to one of four dies. A central line catheter is placed inside a large vein in the chest area and is primarily used for long-term intravenous treatment, lasting for days or weeks. This creates an entry port for microorganisms, increasing the risk of bacterial growth and bloodstream infections. A bloodstream infection is a serious complication and is becoming more difficult to treat due to antibiotic resistance, which is life threatening. Additionally, this causes further financial burden due to prolonged hospitalization. The aim of this project is to find a catheter surface that inhibits the formation of a biofilm by attaching mimics of antimicrobial peptides to the surface (Figure 1) and reduce the number of bloodstream infection incidents.

Methods

The peptides are synthesized by automated Biotage® Initiator+Alstra™ peptide synthesizer using the standard technique of Fmoc solid-phase peptide synthesis. Purification of the peptides are performed by utilizing reverse phase high-performance liquid chromatography (RP-HPLC). Peptides are analyzed by mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR).

The peptides are tested against three laboratory strains in collaboration with DigiBiotics work-package 6 at Department of Medical Biology, UiT.

Result and discussion

Six different peptides were screened against laboratory strains of *S. aureus*, *P. aeruginosa* and *K. pneumoniae* to assess their antimicrobial activity by measuring minimum inhibitory concentration (MIC in µg/mL).

After assessing the activity, the peptides will be attached to the surface to determine inhibition of bacterial colonization.

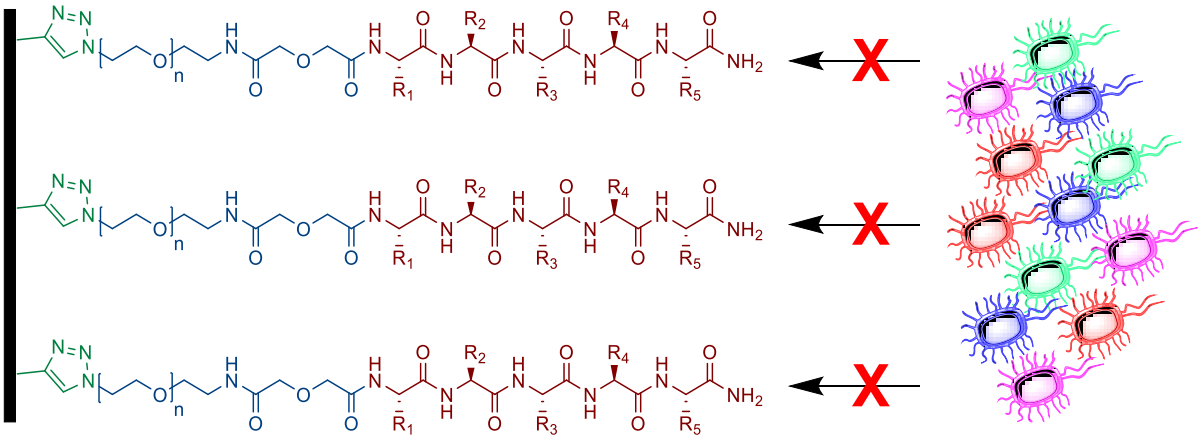


Figure 2. *Mimic of an antimicrobial peptide attached to a catheter surface through a linker to inhibit the formation of a biofilm. R1, R2, R3, R4 and R5 = various amino acid side-chains, n = different length of linker.*

Poster presentations

P1: CRISPR/Cas9 as a potential safe and sustainable solution to the problem of infectious salmon anemia disease in Norway: how far has the research gone?

Author: Okoli, Arinze - GenØk

Introduction:

The current growth and expansion in the Norwegian Atlantic salmon industry is threatened by infectious diseases, in particular the infectious salmon anemia (ISA) caused by the infectious salmon anemia virus (ISAv). The disease is generalized and lethal, characterized by severe anemia, variable hemorrhages and necrosis in several organs. The economic burden of ISA in Norway is enormous: it resulted in a loss of over NOK 1.7 billion in 2016. In 2017, ISA was diagnosed in 14 farming sites compared to 12 sites in 2016; and in 2018 a total of 17 farming sites were diagnosed of ISA. No effective cure against ISA or vaccination strategy against ISAv is currently available. Hence, a new and innovative approach to the ISA problem is required, and CRISPR/Cas9 -a new and novel gene-editing tool that is highly specific, targeted, efficient and cheap, is being put forward as a veritable alternative. Here we present the extent to which this endeavor has gone, and show a road map to an efficient, safe and sustainable application of CRISPR/Cas9 in solving the ISA problem.

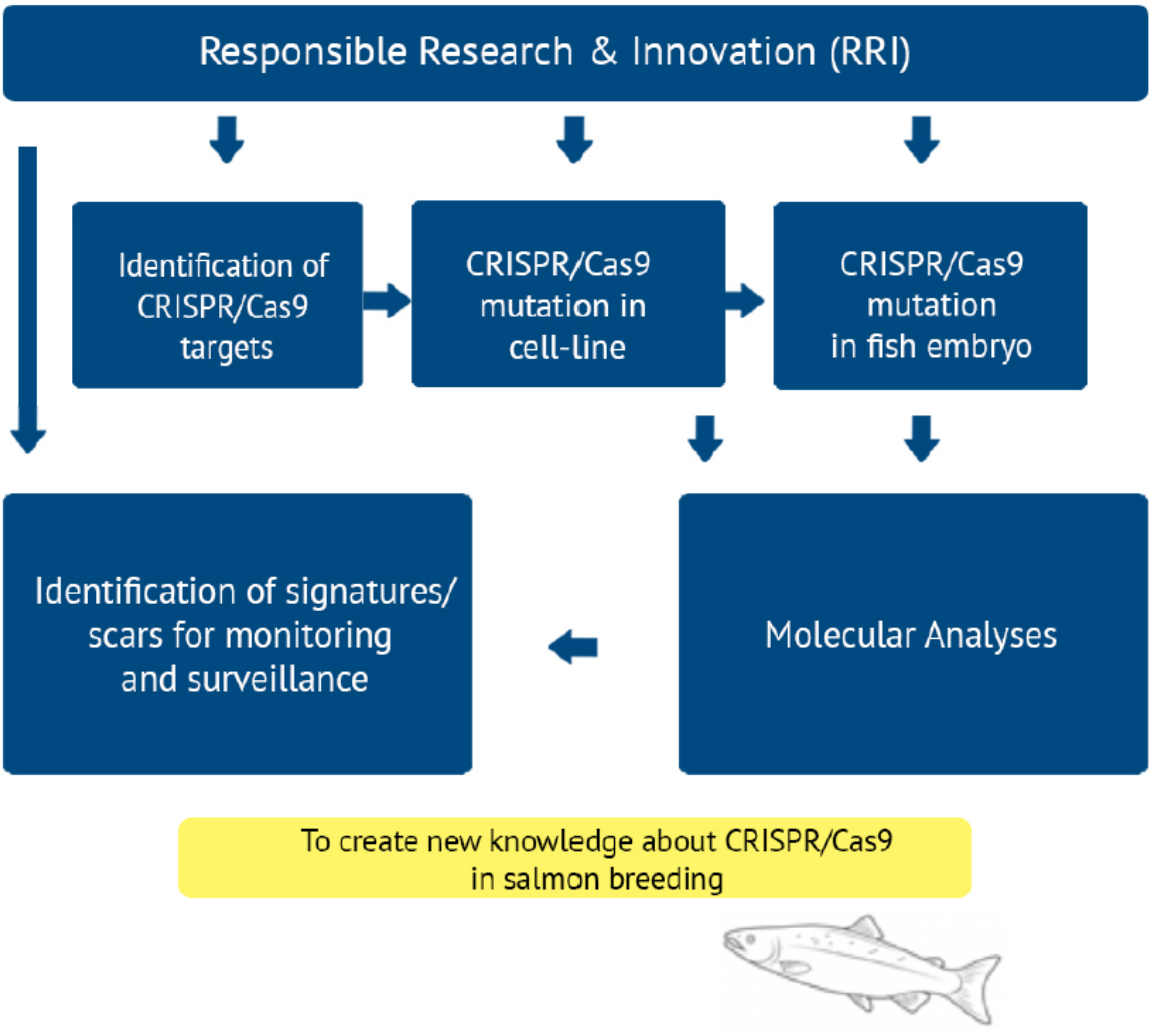
Methods:

A transdisciplinary approach that involved combining stakeholders´ participatory workshop, review of the published literature, and *in-silico* analysis were employed to interrogate how CRISPR/Cas9 can be (and is being) applied as a solution to the problematic ISA disease. Published data on differential gene expression of salmon and/or salmonids-derived cells in response to ISAv infection were analyzed in order to identify potential CRISPR/Cas9 targets. Additionally, other knowledge-needs in the current application of CRISPR/Cas9 in salmon farming, including potential positive and negative impacts on the environment and ecosystem, as well as how the current regulatory landscape in the European Union (EU) may influence both research and application of CRISPR/Cas9 in Norwegian aquaculture were analyzed. Further, aspects in which responsible research and innovation (RRI) can be applied were identified.

Result & Discussion:

The CRISPR/Cas9 technology has been used to produce sterile and albino salmon as a potential solution to the problem of escapee-farmed salmon. However, it is the infectious disease problem of Atlantic salmon that constitutes the major threat to the industry, but no published data exists on CRISPR/Cas9 as a potential solution to the ISA or other infectious diseases. Differentially expressed genes, which contain the 4-O-sialic acid (4-Sias), were identified as good potential targets for CRISPR/Cas9 mutation to achieve ISA-resistant salmon. Off- and on-target effects as well as ecosystem parameters such as new virus strains that may arise if ISA/ISAv-resistant salmon is engineered are data that will be necessary for risk assessment and safety evaluation of the technology as mandated by the Eu and Norwegian regulation. RRI parameters such as openness, transparency, ethics and adherence to sustainability can (and should) be integrated where stakeholders are engaged early, midway and at the end of the research.

Graphical Abstract



P2: An enzyme-constrained genome-scale metabolic model of Streptomyces coelicolor reveals global effects of gene cluster deletion

Author: Sulheim, Snorre - SINTEF Industry

Introduction

Streptomyces coelicolor is the model organism for the *Streptomyces*, a genus of great interest because of their role as a resource for natural products: it is the origin of two-thirds of all clinically relevant antibiotics. *Streptomyces coelicolor* is a natural producer of several antibiotics and a candidate host for heterologous expression of biosynthetic gene clusters, a promising approach for production of novel compounds. Increased interest in this strain and several independent efforts in improving existing genome-scale metabolic models of *S. coelicolor* have created the need for a joint effort in model reconstruction. The response is the development of Sco-GEM, a genome-scale metabolic model of *S. coelicolor* developed and hosted by the community on GitHub. Additionally, we have combined time-series proteomics with Sco-GEM to elucidate how the metabolism changes during a phosphate limited batch fermentation for the two different strains M145 and M1152.

Methods

A genome-scale metabolic model is an overview of the complete metabolic capacity of an organism, i.e. a network of all transport, enzyme-catalysed and spontaneous reactions that can occur in an organism. This overview of reactions is created from the annotated genome and functional annotation of the encoded enzymes and membrane proteins. Consistent and complete annotation of genes, reactions and metabolites in the model enables incorporation of 'omics data which can improve model predictions or aid data interpretation.

By using GECKO, a recently published method which explicitly takes enzyme constraints into account by incorporation of enzyme mass, catalytic coefficients and proteome data, we compare the metabolism of M145 and M1152 during phosphate depletion. While M145 is a wild-type derivate lacking two plasmids M1152 is a further development of M145 where four major biosynthetic gene clusters are deleted.

Result and discussion

Sco-GEM is reconstructed by combining three recent, independent reconstructions of *S.* coelicolor, followed by an improvement of annotations and an analysis of reaction reversibility and transport reactions. This has made Sco-GEM the most complete metabolic reconstruction of *Streptomyces coelicolor* including 1613 genes, 2544 reactions, 2017 metabolites and a Memote score of 77%. By hosting the model and the model development publicly on GitHub we encourage the community to contribute by posting issues, suggesting improvements or actively participate in further development.

Enzyme-constrained versions of Sco-GEM have been created corresponding to different time-points throughout the batch fermentations for both M145 and M1152. Analysis of the time- and strain-specific models have revealed that the deletion of four major biosynthetic gene clusters in M1152 have global effects on the metabolism. This observation will guide further development of *Streptomyces coelicolor* for heterologous expression.

P3: A CRISPR Perturbation Platform for the Detection of Off-target Effects of Gene-Editing Applications in Plants

Author: Zanon Agapito-Tenfen, Sarah - GenØk Centre for Biosafety

Introduction

The programmable clustered regularly interspaced short palindromic repeats (CRISPR) genetic modification tools have the potential to revolutionize agricultural research in the next few years. One important category of assisting technologies in CRISPR gene editing is methods used for detecting and quantifying indels (deletions or insertions). These indels are caused by the repair of CRISPR-Cas9-introduced DNA double-stranded breaks. Although indel detection methods have greatly contributed to the improvement of CRISPR-Cas9 specificity, they still have limitations to cover other genomic sites than the intended one, known as CRISPR's off-target effects. Such analytical constrains are related to the need for PCR amplification, *in vivo* assessment, and the high frequency of somatic mutations and polymorphism of some species. In order to overcome these, we aim at developing a cell-based screening platform to support functional investigations of plant genomes that can reveal how genetic alterations by CRISPR lead to changes in phenotype.

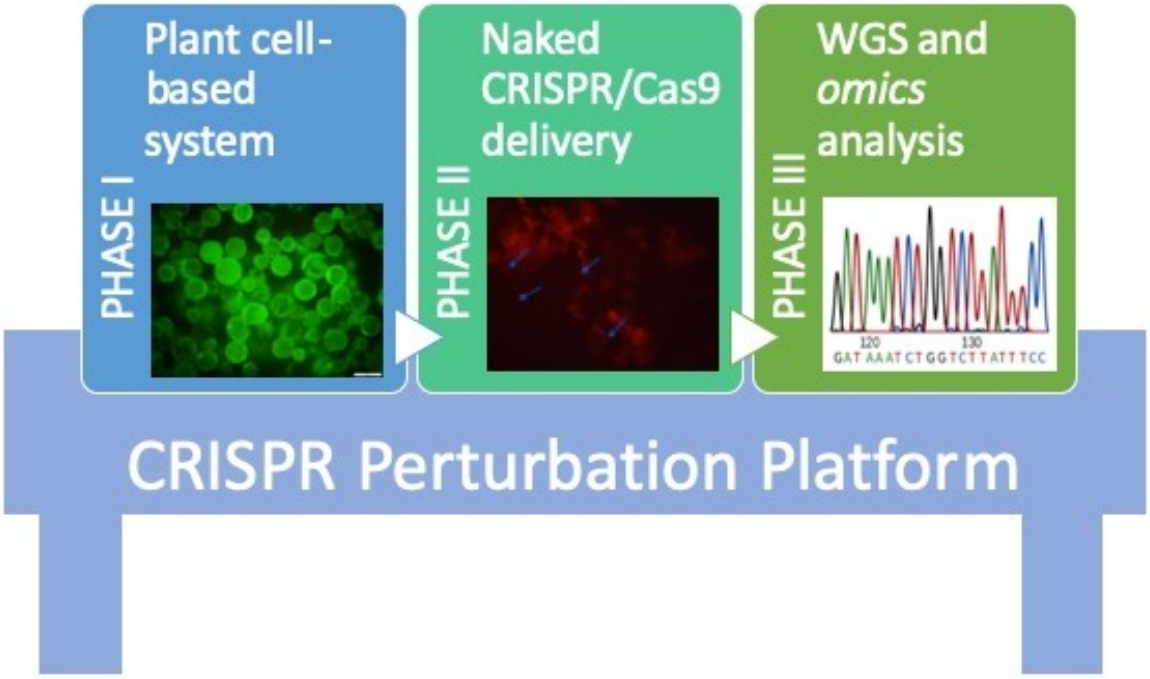
Methods

Platform scientists are developing methods for cell-culture approaches, CRISPR/Cas9 sequencing approaches and untargeted molecular profiling *omics* approaches. In order to isolate the effect of CRISPR/Cas9, cell-culture material is obtained by extracting protoplast cells from mesophyll tissue from seedlings and gene editing performed by PEG-mediated delivery of naked Cas9 as these are considered non-mutagenic approaches. For proof-of-concept purposes, sequencing strategies will be first developed in Columbia-0 arabidopsis, the genome available at Genebank. Further, omics comparative analysis will be performed using off-target site prediction data to investigate the performance of *in silico* off-target analysis. In addition to developing technologies for assessing gene-editing outcomes, the platform also foresees assistance to collaborators in experimental planning and execution, helping them to choose the best model system and most appropriate readout to assess effects of the chosen perturbation.

Results and Discussion

The arabidopsis model system has reached Phase II – CRISPR on-target screens. The results show a range of on-target mutations from +12 to -28 base pairs (bp) with achieved efficiency of approximately 6%. A fluorescent transfection assay has been also established to confirm the internalization of CRISPR/Cas9 ribonucleoprotein complex into cells using fluorescent labeled tracrRNA molecule and microscopy analysis. The next steps at Phase III will include off-target sequencing analysis and *in silico* testing. At Phase III, we will be able to demonstrate the utility of genome-wide analysis of gene editing phenotypes, off-target effects and chromatin structure in gene-edited Arabidopsis and related species. The methods developed here will help improve our basic understanding of CRISPR/Cas9 activities as well as contribute to the detection and identification of gene-editing food products that might enter the European food chain in the near future.

Graphical abstract


PHASE I
Plant cell-based system
PHASE II
Naked CRISPR/Cas9 delivery
PHASE III
WGS and *omics* analysis
120
130
GAT AAAT CT GGTCT TATT TCC
CRISPR Perturbation Platform

P4: 3DLife: Validating new high-throughput methods for 3D cell culture screening

Author: Draget Hoel, Andrea - SINTEF AS

Introduction

Cultivation of cells in a 3D environment is of great interest as it can facilitate *in vivo*-like tissue development, thereby giving more biologically relevant data from cell-based *in vitro* models. As it is vital to understand the interplay between cells and their environment, and how they respond to different physical and chemical conditions, large screening efforts of diverse characteristics is required. It is also necessary to transfer and validate quantitative methods for cell assessment in use for 2D cell cultures, into methods compatible with 3D cell cultures.

Alginate, a linear polysaccharide from seaweed, is an attractive scaffold for cells due to its low toxicity and immunogenicity, together with its ability to form hydrogels at physiological conditions. In addition, it can be tailored to have variable physiochemical properties, e.g. by peptide-grafting or sulfation.

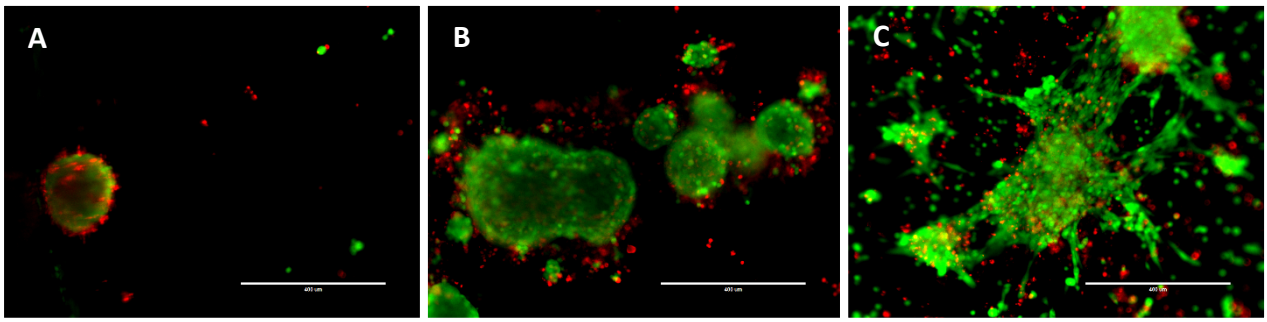
Methods

Different types of fibroblasts have been seeded on top of and encapsulated in various alginate concentrations and modifications: IMR-90 (*lung*), WI-38 (*lung*), HS-5 (*bone marrow/stroma*), HS-27A (*bone marrow/stroma*) and HS-792(C).M (*muscle; connective and soft tissue*).

The cells were dissolved in a citrate-HEPES buffer and mixed with alginate. Alginate gelation was carried out using the slowly hydrolysing glucono-δ-lactone together with $CaCO_3$. After gels had formed (2h), cell culture media was added, and cells were incubated. Viability was evaluated with CellTiter Glo 3D, RNA quantification and Live/dead staining and imaging. The hydrogels were adapted to a 96-well plate format, and robotic protocols have been developed for all handling steps including 3D culture formation, maintenance and preparation for downstream analysis

Results and Discussion

Fibroblasts are the most common mammalian connective tissue cells. They are essential for structure, shape and properties of all tissues in the body, and are thus also highly dependent on interaction with their surrounding matrix material with diverse tissue-dependent characteristics. Different fibroblasts have variable interaction with alginate materials. Sulphated alginate compared with unmodified alginate, gave increased viability for HS-5 cells. Further, RGD-coupled alginate gave both increased viability and changes in morphology (Figure). Such changes can be efficiently screened by our robotic systems and high-throughput screening methods developed for 3D cultures. These platforms provide the means to elucidate the complex interaction between cells and their surrounding environment.



P5: Genome-scale modeling of Auranthiochytrium sp. T66 for increased fatty acid production

Authors: Simensen, Vetle, **Voigt, André** and Almaas, Eivind - NTNU

Introduction

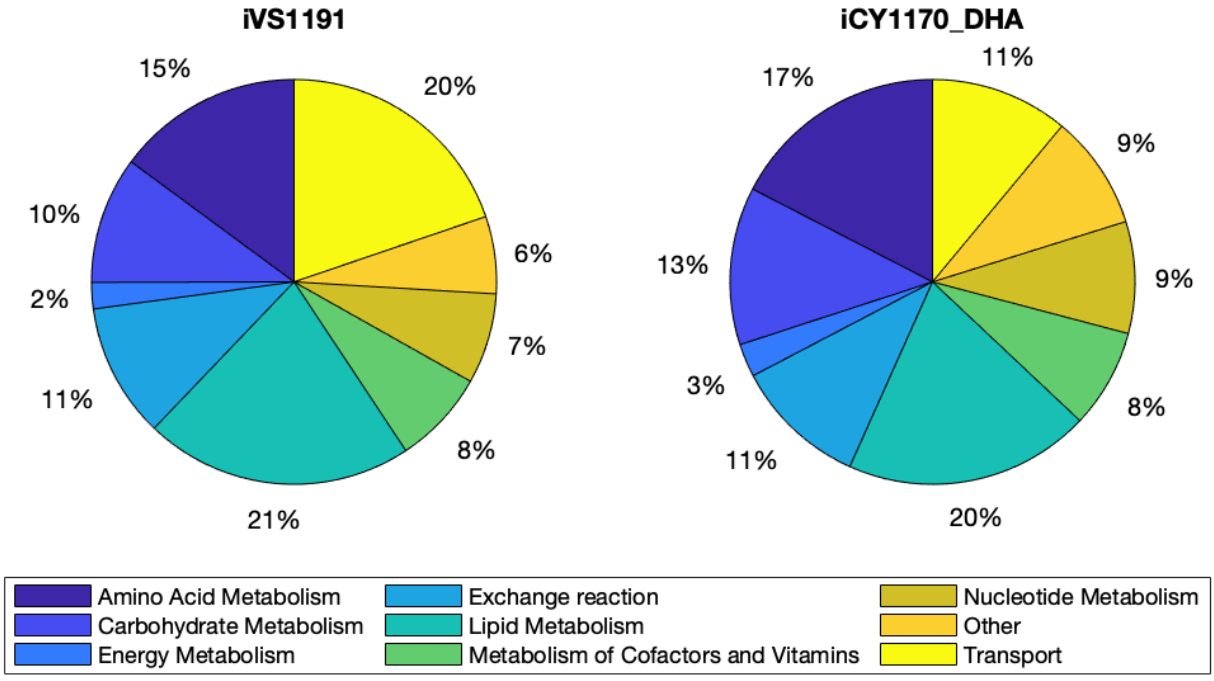
Developing improved fish feed is an important objective for the Norwegian aquaculture industry. The aim of the Auromega project is to engineer strains of Auranthiochytrium sp. T66 with higher concentrations of the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Flux Balance Analysis (FBA) is a well-established method for computational simulation of cell behavior, and enables us to identify promising bio-engineering strategies for a given objective. However, in order to be effective, FBA requires a high-quality reconstruction of the relevant organism with a comprehensive overview of its metabolic capabilities.

Methods

Using an already published GSM of a closely related strain, we used automated tools to reconstruct a draft model of Auranthiochytrium sp. T66 using genomic data. The model was then manually curated and modified by adding known capabilities missed by the automatic reconstruction, as well as gap-filling techniques to correct blocked pathways.

Result and discussion

The generated model consisted of 2093 unique metabolic reactions, 1668 etabolites, and 1191 associated genes. Simulated gene essentiality predictions on carbon-limited minimal medium revealed a robust and adaptable metabolic network, able to grow at sub-optimal or optimal growth phenotypes for around 81% of all single-gene knockouts. Using the OptKnock algorithm, multiple double-reaction knockout strategies were proposed for the increased production the key lipid precursors malonyl-CoA and NADPH.



P6: Measuring of the biomass composition for modelling purposes

Author: Schulz, Christian - NTNU

Constraint-based modeling and analysis has become a paradigm of systems-biology investigations of genome-scale microbial metabolism. For all such investigations, the assumed microbial biomass composition is a fundamental part with the potential of significantly affecting the computational results and predictions. For example in the method of Flux Balance Analysis (FBA), the biomass composition is used as an objective function that is optimized. Consequently, high-quality measurements of the biomass composition will have a major influence on, e.g. the ability for growth-, knockout-, or metabolic engineering-predictions to be in accordance with wet-lab experiments.

However, a large number of genome-scale metabolic reconstructed models do not contain a measurement-based biomass composition. Moreover, many of the implemented biomass compositions are inherited from previous models or are estimated based on (inherited) biomass compositions from “closely related organisms” if no other models for the organism exist. Furthermore, the majority of published metabolic reconstructions do not contain different biomass compositions for different environments, even though an organism’s biomass may change dramatically (e.g. certain *Pseudomonas* strains can accumulate polyhydroxyalkanoates up to 80% of dry biomass). Such changes in composition are not covered by a single biomass formulation. Consequently, predictions for different environments will not be of as high a quality as for the environment the biomass composition was developed in.

Here, we present a combined effort of computational and experimental expertise to approach this problem. We have used a wide range of techniques, from spectroscopy and HPLC to MS analyses to determine the biomass composition of different bacteria, for example *E. coli*. Therefore, we measured DNA, RNA, protein (amino acid composition), lipids (lipid composition), and carbohydrates (carbohydrate composition) of lyophilized biomass from batch cultures.

We intend to further develop and broaden this pipeline of experimental approaches and protocols, so that we may study species such as *Saccharomyces*, *Pseudomonas*, and *Bacillus* to name a few, as well as bioreactor experiments to determine growth-, uptake-, and excretion rates in specified environments and phases of the organisms.

P7: Novel redox enzyme systems for lignocellulosic biomass conversion

Authors: **Stepnov, Anton A.,** Votvik, Amanda K., Forsberg Zarah, Røhr Åsmund K., Eijsink, Vincent G.H.
Faculty of Chemistry, Biotechnology and Food Science, NMBU - Norwegian University of Life Sciences.

Introduction
Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes that catalyze the oxidative depolymerization of recalcitrant polysaccharides. By utilizing powerful redox chemistry, LPMOs are able to disrupt highly crystalline regions of their substrates that are normally inaccessible to classical hydrolytic enzymes. Thus, LPMOs provide a significant boost to the overall efficiency of biological or industrial lignocellulosic biomass conversion. Despite the large amount of data published in recent years, bacterial LPMOs are still underexplored and their natural redox partners in biomass-degrading enzyme systems remain unknown. In the OxyMod project we will unravel and engineer bacterial redox enzyme systems for biomass conversion. Here we report the results of *in silico* mining for novel LPMOs in a unique dataset of 2000 actinobacterial genomes as well as in public databases.

Methods
The initial analysis of the metagenomic dataset using an HMM (hidden Markov model) profile of AA10-type (i.e., bacterial) LPMOs revealed 2826 putative enzyme sequences, which were used to build a shortlist of 118 project candidates. The enzymes were selected on the basis of several criteria, including novelty (sequence identity) and phylogenetic position (see Fig. 1).
Nine shortlisted LPMO genes were codon-optimized for expression in *E. coli*, synthesized and cloned using three expression vectors (pNIC-CH, pRSET-B, pET-26b) and three types of signal peptides (native, CBP21 or PelB signal sequences). The first batch of candidates comprised six putative cellulose oxidizers and three LPMO-like proteins with unpredicted substrate specificity. Four out of nine putative LPMOs were produced in *E. coli* and then isolated from periplasmic extracts by ion-exchange and size-exclusion chromatography.

Result and discussion
Oxidative activity towards cellulose was confirmed for three enzymes (AA10-06, AA10-07 and SScLPMO10B) by MALDI-TOF MS and these LPMOs are currently being characterized in detail. The AA10-02 putative LPMO did not show any activity towards amorphous and microcrystalline cellulose, β-chitin and a variety of hemicellulosic substrates. However, the protein stability was clearly affected by the presence of copper, which indicates that the active site is intact and may be functional. This notion was further supported by a hydrogen peroxide production experiment, showing formation of H_2O_2 by the reduced protein, which is considered a characteristic LPMO feature. Thus, AA10-02 may represent a LPMO with an unknown type of activity.
This work is a part of OxyMod project within Center of Digital Life Norway, and is funded by the Research Council of Norway (project 269408).

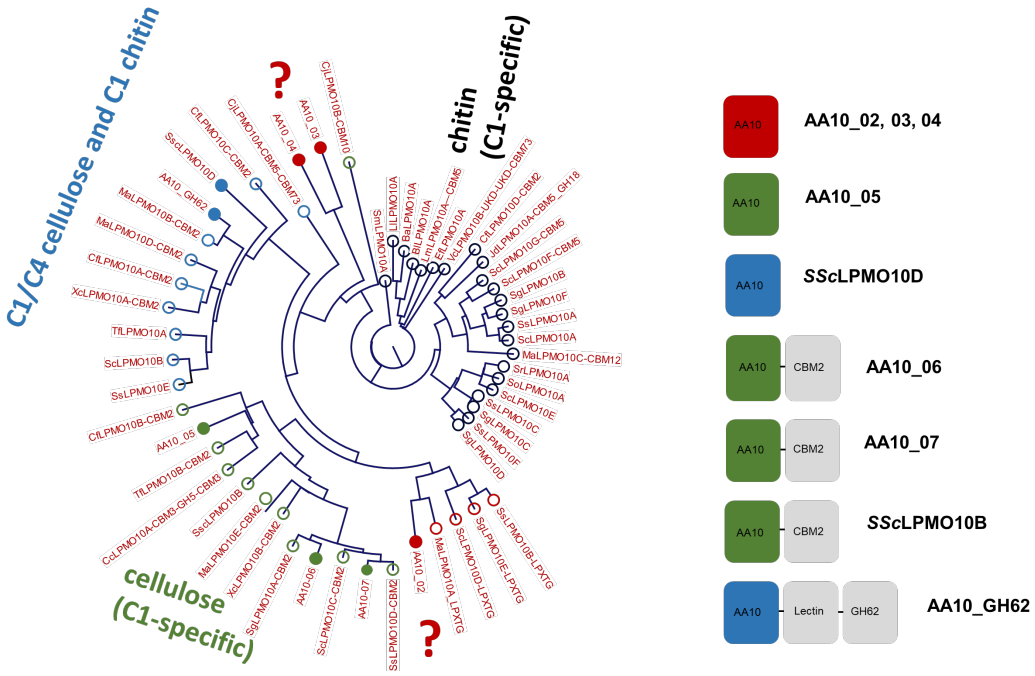
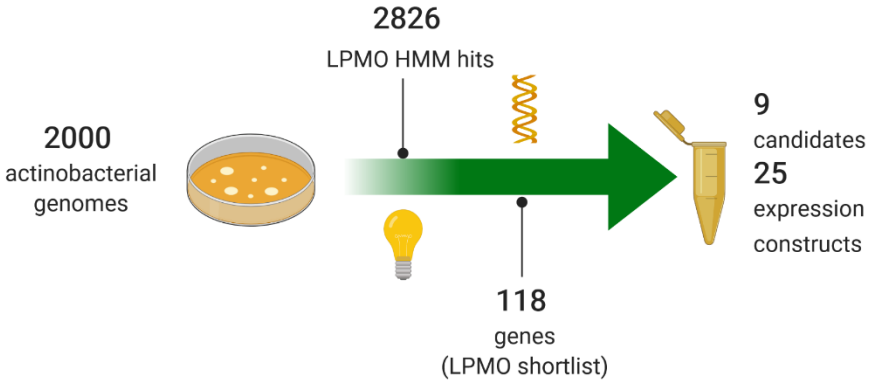


Figure 1. Phylogenetic classification of LPMO candidates. The phylogram shows the sequence relationship of nine project candidates to reference enzymes with their predicted substrate specificities. The candidate LPMOs are marked with filled circles. The substrate specificity is color-coded as follows: black – chitin C1-oxidizers; green – cellulose C1-oxidizers; blue – cellulose C1/C4-oxidizers; red – unknown substrate specificity. The domain architecture of the selected LPMOs is shown in the right panel. CBM2 – family 2 cellulose binding module; GH62 – family 62 glycosyl hydrolase domain.

Graphical abstract:



P8: Platform development for *In-vivo* biopolymer engineering

Author: Schmid, Jochen - Department of Biotechnology and Food Science, NTNU

Introduction

How will we meet the globally growing demand for biobased and biodegradable “green” polymers when it comes to the replacement of fossil based high performance polymers? For decades, microbial polysaccharides have been used in a vast amount of applications such as food, feed, pharmaceutics as well as technical ones. However, a lack of knowledge regarding structure-function relationship as well as tailoring chemical structures render this approach limited to this day. Therefore, a platform to engineer microbial polysaccharides for the targeted application in various fields is the aim of this project. The design of tailored polysaccharides in combination with structure-function analysis will enable high-throughput engineering of microbial exopolysaccharides (EPS) in two selected chassis organisms by use of approaches from synthetic and systems biology.

Methods

The scope of the project is the development of two chassis organisms for the heterologous expression of EPS encoding gene clusters. Based on tailored CRISPR-Cas systems the Gram(-) *Xanthomonas campestris* and the Gram(+) *Paenibacillus polymyxa* EPS producing strains will be modified towards heterologous EPS production. Based on a cluster replacement approach a broad variety of EPS encoding gene clusters will get accessible for modification by use of the established molecular tools, and the functional analysis of the different genes and enzymes can be performed. By that, several tailored chemical structures can be realized which will enhance the insights in structure-function relationship, by creating structures just differing in single sugar monomers. In addition, and a library of EPS-biosynthetic genes will be set-up to represent the base for a combinatorial assembly of synthetic EPS soon. This library will be filled up by intensive mining of genome databases, to identify suitable parts for the synthetic EPS-biosynthetic machineries. Finally, a physicochemical analysis will show the suitability of these novel polysaccharide variants in specific applications.

Result and discussion

Based on a tailored CRISPR-Cas system, the native EPS-biosynthesis encoding operon (34 kb) of *P. polymyxa* was targeted and more than 35 different knock-out variants were created to functionally analyze the different genes. Next to knockout variants gene replacement and integration of larger nucleotide sequences (> 5 kb) was successfully shown. For *X. campestris* several knock-out variants of the xanthan biosynthesis encoding gene cluster were realized by use of a suicide plasmid and several variants of modified xanthan were produced. Just recently a specific Xanthomonas CRISPR-Cas system was designed and is under evaluation. In sum there are many new polysaccharide variants available which will be analyzed in detail for their physicochemical behavior.

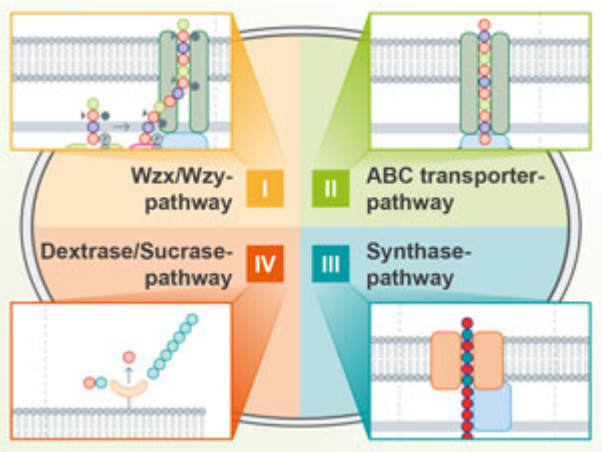


Figure: *Graphical abstract*

P9: Fabrication and characterization of silicon photonic sensor

Author: Yadav, Mukesh - Department of Electronic Systems, NTNU

Introduction:
In recent years, there has been growing interest in optical waveguide-based sensors because of the usefulness of optical waveguide sensors in detection of chemical, biological and physical parameters. For example, ring resonator, Mach-Zehnder interferometer, and spectroscopy-based waveguide sensors have shown applicability in detection of specific enzymes, proteins, etc. Most of the optical waveguide sensors are based on evanescent field sensing. In evanescent field sensing, exponentially decaying electromagnetic fields interact with the analyte and causes changes in refractive index, phase, intensity and polarization. These optical properties can be detected with different sensor designs. We present here fabrication and characterization of an integrated ring resonator-based waveguide sensor for biochemical detection.

Method:
To fabricate a silicon ring resonator (RR) waveguide sensor, a silicon-on-insulator (SOI) platform was used. Firstly, 220 nm thick layer of amorphous silicon was deposited on a 1 um thin thermal-oxide wafer by using plasma enhanced chemical vapor deposition (PECVD). Electron beam lithography (EBL) was performed on the SOI-chip to generate a ring resonator pattern with radius of 15 um. EBL (Elionix ELS-G100) was then followed by inductive coupled plasma reactive-ion etching (ICP-RIE) for anisotropic dry etch to define the silicon waveguide structure. In order to characterize a photonic chip, polydimethylsiloxane (PDMS) based microfluidics was fabricated using soft lithography. The PDMS microfluidic was integrated with the photonic chip to perform characterization. For optical characterization, a tunable laser source (Thorlabs TLK-1550M) was coupled to the photonic chip using a tapered fiber and the output power was measured using a photodetector (Thorlabs DET10C2). For controlled fluid flow, tubings from the PDMS microfluidics were connected to the syringe pump (Havard Pump 11 pico plus elite).

Results and discussion:
To study the response of our ring resonator sensor, output power spectra were measured with a Labview program. Output spectra from the RR were further analyzed using Matlab scripts to perform Lorentzian fit. Fig.1, shows the RR spectral response and its Lorentzian fit. The RR responsivity was analyzed by measuring the relative resonance shift with different concentrations of salt water. As shown in Fig. 2, a relative resonance shift of *110 pm/% salt concentration* was measured for the RR with 15 um radius. From these measurements, we found that the sensor response is linear with the analyte concentration. The fabricated ring resonator can be useful for biosensing applications, since they require high sensitivity and small size.

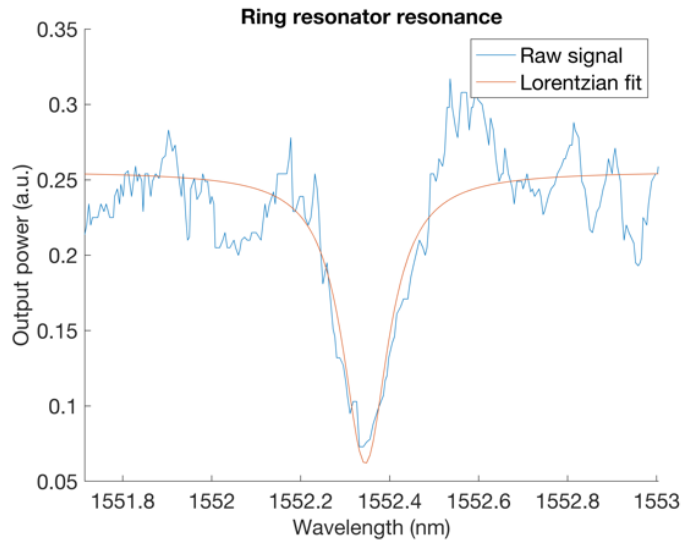


Figure 3. Ring resonator response (wavelength vs output)

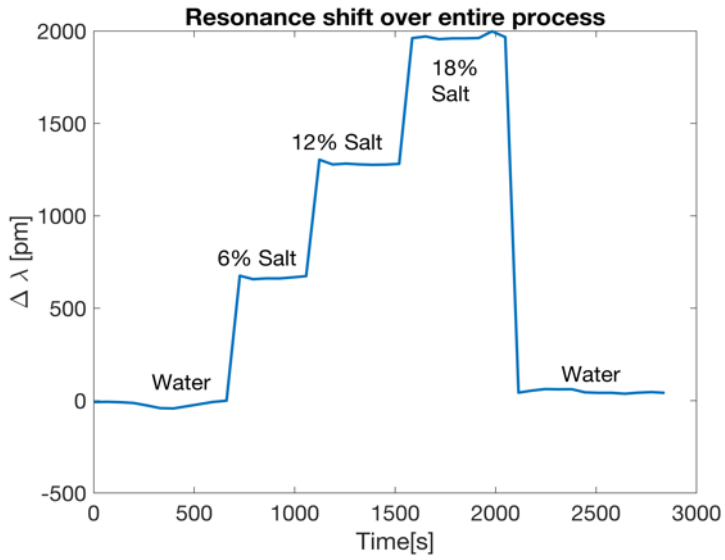


Figure 4. Relative resonance shift vs conc. of salt

P10: Unravelling the role of the phosphatase PP2A-B56 in the stabilization of the G1 phase of the cell cycle in *Schizosaccharomyces pombe*

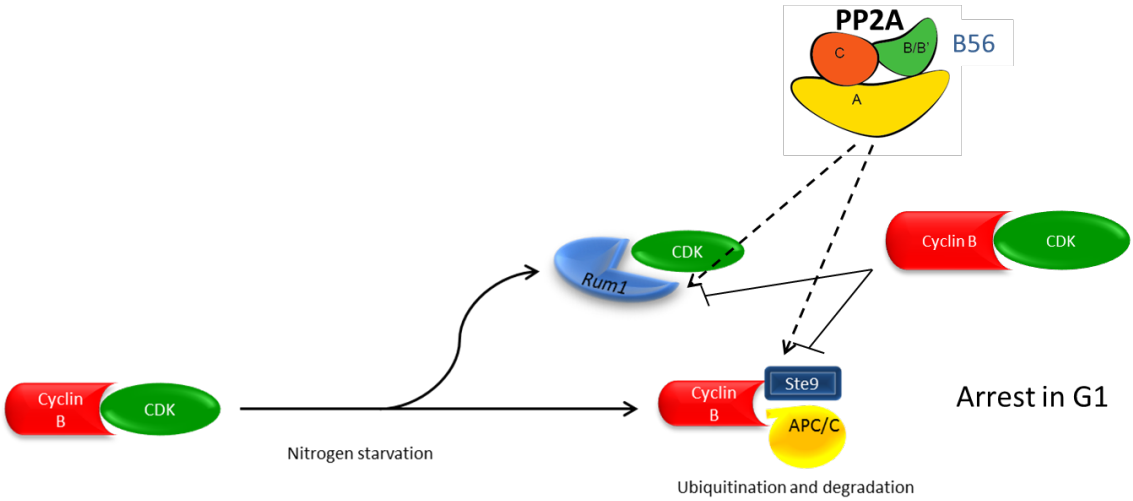
Author: Stonyte, Vilte - NCMM, UiO

A tight regulation of a high number of signalling pathways is required to coordinate cell growth and cell cycle progression with the availability of resources in a changing environment. During cell proliferation, cells sense the abundance of nutrients to make the decision of dividing into two cells. This process has to be highly regulated and failure in its regulation can promote uncontrolled growth.

In *S. pombe*, nitrogen starvation promotes cell cycle arrest in G1 phase that leads to activation of the transcriptional program to differentiate sexually. To prevent the cell cycle progression and arrest in G1, the cell has to maintain low CDK-Cyclin activity. In *S. pombe*, this is achieved through the accumulation of the CDK inhibitor Rum1 and the activation of Ste9 (activator of the Anaphase Promoting Complex). This regulation is very intricate due to the presence of feedback loops by which CDK activity can inhibit these negative regulators. Given this phosphorylation-based control, we hypothesize the implication of phosphatase activity in this process.

Mutant strains used in this study were created by targeted mutagenesis, deleting specific genes, generating conditional mutants, introducing tags. To identify and confirm the phosphatase involved in this mechanism, mating efficiency was compared by quantifying zygote and tetrad formation, and ability to arrest in G1 was assessed using flow cytometry. Immunoblot analyses were carried out to check functionality of nutritional signalling pathways, behaviour of cell cycle regulators and genetic interactions among proteins of interest. Protein purification and co-immunoprecipitation assays lead to identification of direct interaction.

Our genetic studies show that PP2A B56-Par1 is the main phosphatase involved in counteracting CDK-Cyclin B activity facilitating the arrest of the cells in G1. Moreover, the activity of Par1 is necessary for stabilization of the CDK inhibitor Rum1, and it promotes the dephosphorylation of Ste9. Finally, using biochemical assays, we observe a direct interaction between Rum1 and Par1, suggesting Rum1 as a substrate of PP2A. The pivotal role that PP2A plays counteracting CDK phosphorylation has significant consequences for cell differentiation, which in all eukaryotes can only occur during G1 phase. This role could be extended to other situations that require stalling of cell cycle progression through G1 and therefore constitutes an important element of CDK control.


PP2A
C
B/B'
B56
A
CDK
Rum1
Cyclin B
CDK
Cyclin
B
CDK
Nitrogen starvation
Cyclin
B
Ste9
APC/C
Ubiquitination and degradation
Arrest in G1

P11: Alginate hydrogels with tailored mechanical and biological properties for 3D fibroblast culture

Author: Zaytseva-Zotova, Daria - NTNU

Introduction

Cells of the human body normally grow in a three-dimensional (3D) environment surrounded by extracellular matrix (ECM). The ECM is essential for normal cell functioning: if cells lack 3D ordering and networking they can lose their tissue-like characteristics and phenotype. Alginate is an attractive polymer for fabrication of hydrogels that mimic native ECM. Alginate hydrogels have been shown to have in general good biocompatibility, tunable physical and mechanical properties, and they can be fabricated under physiological conditions allowing gentle entrapment of living cells. But alginates do not contain any specific binding sites for cell attachment.

The aim of our work is to develop hydrogels with mechanical and biological properties tailored for the specific needs of a given cell type or cell system, and in this case fibroblasts.

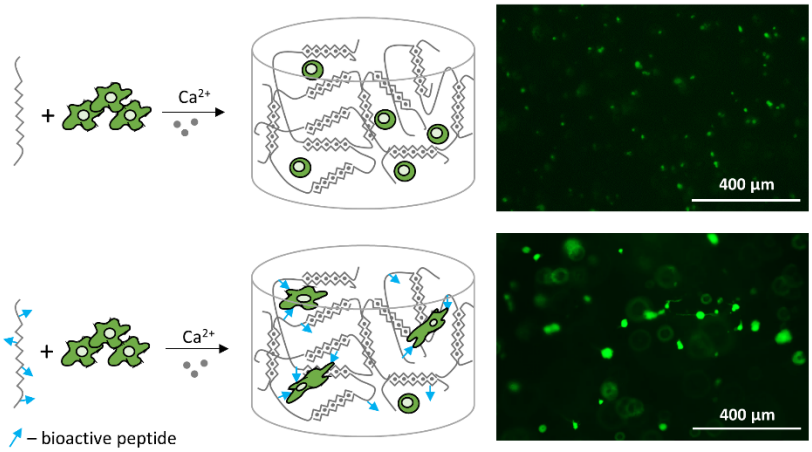
Methods

Bioactive peptides (IKVAV, YIGSR and GRGDSP), that mimic cell binding motifs of laminin and fibronectin, were grafted to alginates (F_G 0.68, M_W 240-300 kDa) using previously described method (Dalheim et al 2016). Hydrogels were crosslinked with calcium ions using internal gelation method – a well-known strategy for formation of soft gels with homogenous and reproducible structure (Draget et al 1990). Gelation kinetics and mechanical properties were characterized using oscillatory rheometry. For cell studies, hydrogels were casted in 96-well plates according to the protocol developed by us for robotic handling. Human fibroblasts were cultured on or within the hydrogels for up to 10 days. Every day cells were labeled using Live/Dead stain and examined under fluorescence microscope.

Result and discussion

In this work we created a library of alginate hydrogels having mechanical properties within the range typical for soft tissues (G' 10-1500 Pa) and functionalized with three model bioactive peptides. Gel formation procedure was optimized with regards to desired gelation kinetics for compatibility with the robotic system for future high-throughput screening of cell responses. Our preliminary results showed that the viability and morphology of fibroblasts depended on both mechanical and biological properties of the developed hydrogels, e.g. human lung fibroblasts (IMR90) favored softer gels functionalized with GRGDSP sequences (see graphical abstract).

This work is a part of 3DLife project within Center of Digital Life Norway, and is financed by the Research Council of Norway (project 269273).


Ca2+
400 µm
Ca2+
400 µm
– bioactive peptide

P12: Discovery and characterization of novel redox enzymes from Trondheim fjord's actinobacteria strain collection for lignocellulosic biomass degradation

Author: Nguyen, Giang Son - SINTEF Industry

Introduction

Understanding enzymes and enzyme systems involved in lignocellulosic biomass degradation is the central topic of the OXYMOD project, funded by the Center of Digital Life Norway. Beside enzymes that are active against cellulose, redox enzymes such as laccases and peroxidases contribute to the process of breaking down lignin fraction. From long term bioprospecting activities by SINTEF and NTNU, a unique collection of actinobacteria strains has been established and 1200 strains have been sequenced using Illumina shotgun sequencing. The actinobacteria phylum has been known as a rich source of enzymes for lignin degradation and therefore is an attractive target for *in silico* mining of novel redox biocatalysts.

Methods

A customized workflow of data mining based on profiles HMM, multiple sequence alignment, and sequence similarity network has been developed and used for mining of new laccases and peroxidases from the database of 1200 actinobacteria genomes. The detected hits were further manually curated to have a shortlist of sequence candidates which were subsequently sent for gene synthesis and subcloned in expression vectors. The candidates were expressed, purified and the activity of the enzymes were tested against model substrate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS). The laccases and peroxidases with confirmed ABTS activity were subsequently screened against a panel of small phenolic compounds as well as subjected to physiochemical characterization (pH optimum and stability, temperature stability).

Result and discussion

For mining of the new laccases, a custom profile HMM was created based on the sequences of two-domain small laccases, SLAC-like family (Hfam39), obtained from Laccase and Multicopper Oxidase Engineering Database (LCCED). Four out of seven new laccase candidates were expressed in the soluble form and showed activity against ABTS after purification. Two enzymes, namely P06-A08 and P20-F12 appeared to be thermostable (activity was maintained even after two hours incubated at 80 °C). The pH optimum of the enzymes was identified in the range of pH 3.8 and 4.2 with the sharp decline of activity from pH 5 and forward. Among compounds tested for substrate scope study, activities against ferulic acid, sinapic acid, catechol, and 3,4-dihydroxybenzoic acid were confirmed.
Most of identified peroxidase hits from the strain collection belong to the Dyp-type family (dye de-colourising peroxidase). Like the new laccases, four peroxidases were active against ABTS after purification and have a pH optimum in the limited range of 3.0 and 3.4. The de-colourising activity was confirmed using commercial dyes, Remazol Brilliant Blue R and Reactive Blue 5.

P13: Biodiscovery of Antimicrobial Compounds from Arctic Marine Microorganisms

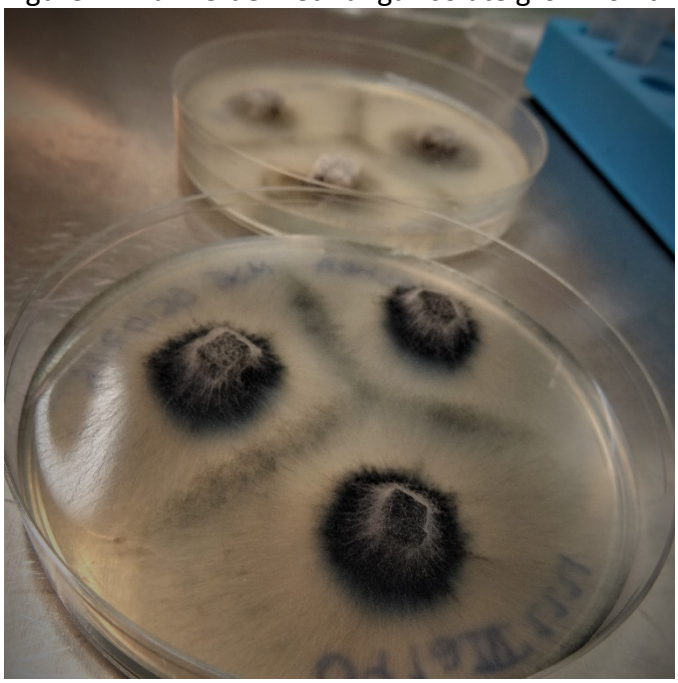
Author: Jenssen, Marte - UiT the Arctic University of Norway

Introduction:
Antimicrobial resistance is a rapidly growing problem in all parts of the world. How can marine microorganisms help us solve this? Natural products are important in the discovery and development of drugs. Due to difficulties in sampling and cultivation, marine microorganisms have not been as well investigated as their terrestrial counterparts. In DigiBiotics, we aim to use these marine microorganisms to find compounds that can be the antibiotics of tomorrow.

Methods:
The microorganisms are cultivated in different media in an attempt to trigger the activation of different biosynthetic gene clusters, and thereby triggering the production of different secondary metabolites. These metabolites are extracted and tested for antibacterial activities and the ability to inhibit biofilm formation. HPLC-MS is then used to point out possible candidates that could be responsible for the activity. A preparative HPLC-MS system is used to isolate the compounds, and their structure is elucidated by MS and NMR.

Results:
Active fractions have been identified in the antibacterial screening of the marine derived fungi. Several candidates in these fractions are nominated for further isolation. Two compounds are now in the process of being structurally elucidated before further bioactivity testing.

Figure: A marine-derived fungal isolate grown on two different solid media.



P14: Transdisciplinarity in Norwegian Biotechnology Research

Author: Hesjedal, Maria Bårdsen - NTNU

Introduction
In 2012 the Norwegian Research Counsel (RCN) launched a programme called BIOTEK2021, and as a part of this the strategic initiative "Digital Life – Convergence for Innovation". A central element in the digital life initiative was the formation Digital Life Norway (DLN). Both in the RCN's strategic initiative and in DLN transdisciplinarity is highlighted as a crucial feature, together with Responsible Research and Innovation (RRI). In the DLN's project calls to the Norwegian biotechnology community, transdisciplinarity was demanded of the project applications. In my project I am interested in
How to improve the understanding of inter-relationships between science and society in biotechnology projects.
To explore how such knowledge can facilitate the interactions between science and society.
Exploring implications of increased inter- and transdisciplinarity in biotechnology.

Methods
This is a qualitative research project, and the main research methods are interviews and participant observation in three DLN projects, respectively on tissue engineering, environmental toxicology and the mechanisms of brain diseases. Though they are all interdisciplinary projects, they have different ways of approaching and managing their collaboration.

Result and discussion
I will compare the three projects and discuss them in the context of DLN and Norwegian biotechnology. This will help us gain insights in how the demands for inter- and transdisciplinarity is met and managed in the projects, how this is experienced by the project participants, what works and does not work, as well as challenges and barriers in Norwegian biotechnology.

TRANSDISCIPLINARITY IN BIOTECHNOLOGY

Introduction

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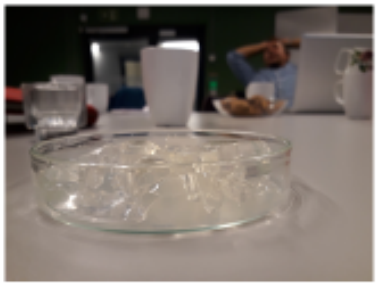
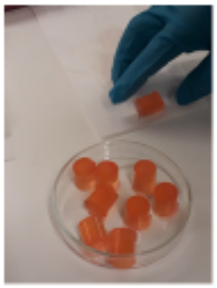




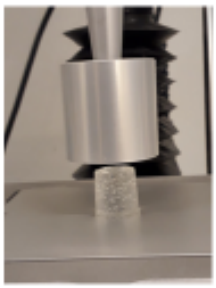
Exploring implications of increased inter- and transdisciplinarity in Norwegian biotechnology

Methods

This is a qualitative research project, and the main research methods are interviews and participant observation in three DLN projects, respectively on tissue engineering, environmental toxicology and the mechanisms of brain diseases. Though they are all interdisciplinary projects, they have different ways of approaching and managing their collaboration.

Discussion

I will compare the three projects and discuss them in the context of DLN and Norwegian biotechnology. This will help us gain insights in how the demands for inter- and transdisciplinarity is met and managed in the projects, how this is experienced by the project participants, what works and does not work, as well as challenges and barriers in Norwegian biotechnology.



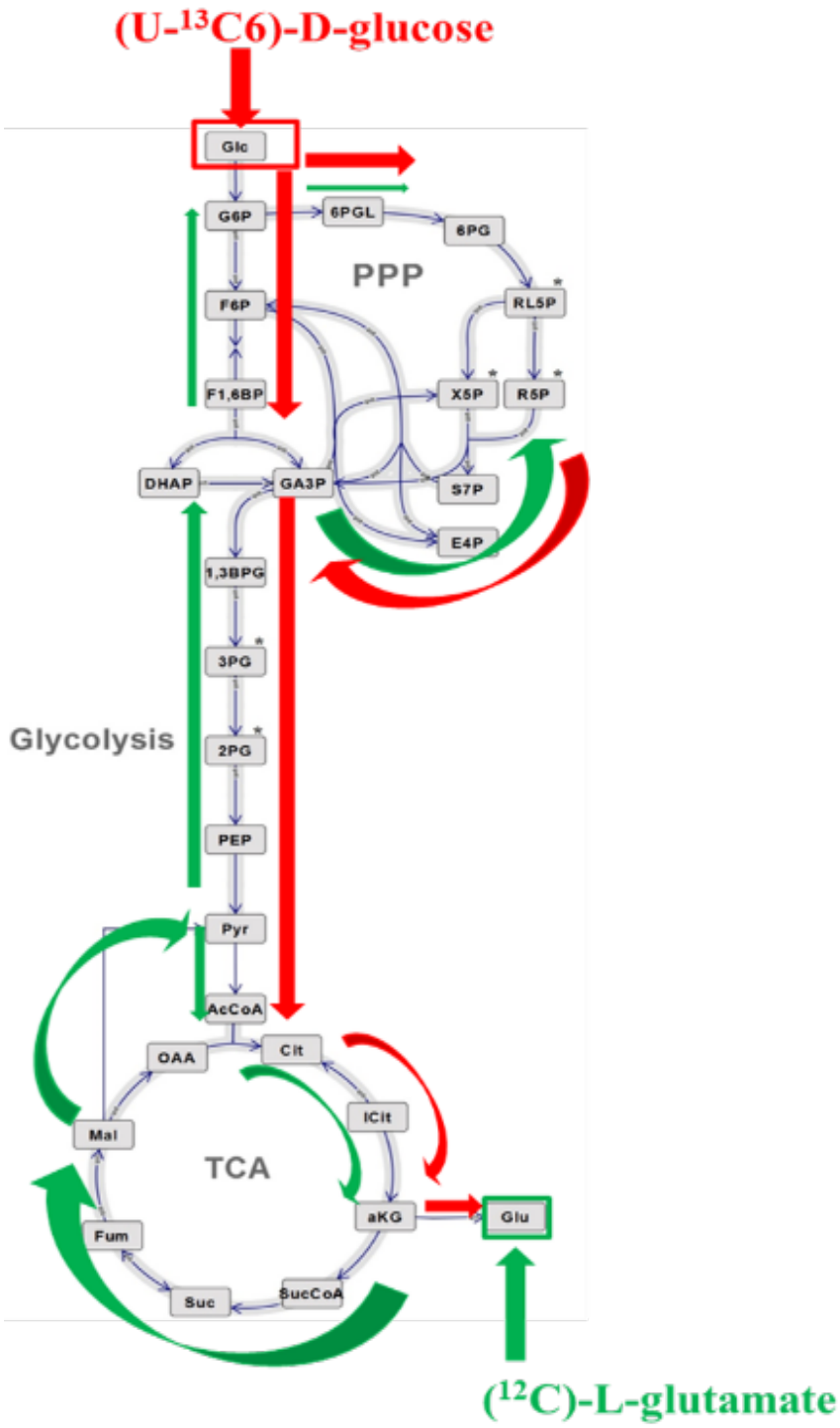
P15: Studying the metabolism of *Streptomyces coelicolor* during antibiotic production using ^{13}C-isotope-labeling experiments

Author: Kumar, Kanhaiya - NTNU

Introduction:
The presented work relates to our efforts to develop optimized *Streptomyces* 'Superhost' strains to act as microbial cell factories for a more efficient discovery of clinically important novel bioactive natural products by using Systems and Synthetic Biology approaches. This project has a very high importance because of increasing antibiotic resistance among pathogenic bacteria and often futile attempts to discover novel and potent antibiotics. *S. coelicolor* A3(2) is well-known microorganism to produce different types of antibiotics and is considered suitable to create 'Superhost' strains because of the available knowledge of genome sequence. One important experimental input to this task is deciphering the participation of important metabolic pathways for antibiotic production and generation of high-resolution quantitative metabolite profiles of the *S. coelicolor* host production strains.

Methods:
S. coelicolor A3(2) M145, its non-antibiotics producing mutant strain *S. coelicolor* A3(2) M1152 and chloramphenicol (CP) producing *S. coelicolor* A3(2) M1581 (a derivative of *S. coelicolor* A3(2) M1152) were used in this study. Microorganisms were cultivated in controlled bioreactors using medium designed to provide either L-glutamate or phosphate limitation. Glucose and other extracellular components were measured using HPLC. Metabolites samples were collected at different stages of cultivation and were processed to quantify Intracellular metabolite pools using several MS/MS based methods; i.e. two UPLC-MS/MS methods for amino acids and organic acids, a capIC-MS/MS method for nucleotides, sugar phosphates and other phosphometabolites. D-glucose (U-^{13}C6) and naturally available L-glutamate were used in the ^{13}C-isotope-labeling experiments.

Results and discussion:
Both L-glutamate or phosphate limitation triggered antibiotics production at a defined time in *S. coelicolor* A3(2) M145. The empty strain of *S.coelicolor* A3 (2) M1152 had a prolonged CO_2 evolution phase as compared to wild type strain. ^{13}C-isotope-labeling experiments showed the degree of participation of carbon from L-glutamate and D-glucose in different metabolic pathways and in synthezied antibiotics (Fig.). L-glutamate enters metabolic pathways through tricarboxylic acid cycle (TCA) and transfers its carbon into glycolytic pathway and then into pentose phosphate pathway mostly through glyceraldehyde-3-phosphate. Isotopic patterns of citrate show metabolites flooding situation in different metabolic pathways especially in case of non-antibiotic producing strain as along with D-glucose, L-glutamate carbon re-enters into TCA cycle from glycolytic pathway.


(U-13C6)-D-glucose
Glc
G6P
6PGL
6PG
PPP
F6P
RL5P
F1,6BP
X5P
R5P
DHAP
GA3P
S7P
E4P
1,3BPG
3PG
Glycolysis
2PG
PEP
Pyr
AcCoA
OAA
Cit
ICit
Mal
TCA
aKG
Glu
Fum
Suc
SucCoA
(12C)-L-glutamate

P16: Synthesis of membrane targeting compounds - a promising target for new antibiotics

Author: Kristoffersen, Tone - The Arctic University of Norway, UiT

Introduction

The emerging antimicrobial resistance crisis threatens the standard of care in modern medicine and everyday life. An important contribution to combat the crisis is the development of new antibiotics. The DigiBiotics consortium aims to find, design and develop novel antibiotics inspired by marine Arctic natural products. The compounds of interest are in the "middle-space" region, with molecular weights ranging from 600 to 2000 Da. These middle-spaced molecules have been largely unexplored due to unfavorable drug properties, however, they have great potential to interact with targets not widely used today, such as the bacterial membrane. This project aims to synthesize and explore promising antibacterial compounds and search for more druggable compounds by diversifying the natural product structure. As a starting point, known membrane active amphipathic peptides will be synthesized using an automated microwave assisted standard solid-phase peptide synthesizer. These peptides will form the foundation for the development of state-of-the-art tools for studying peptide-membrane interactions, through both biological essays and computational methods. These state-of-the-art tools will ease the drug development process in the future lowering both money and time costs.

Methods

New compounds are formed using standard organic synthetic methods, and purified by standard purification methods such as flash- and high-performance liquid chromatography. Structure elucidating are performed using nuclear magnetic resonance (NMR) and mass spectrometry (MS).

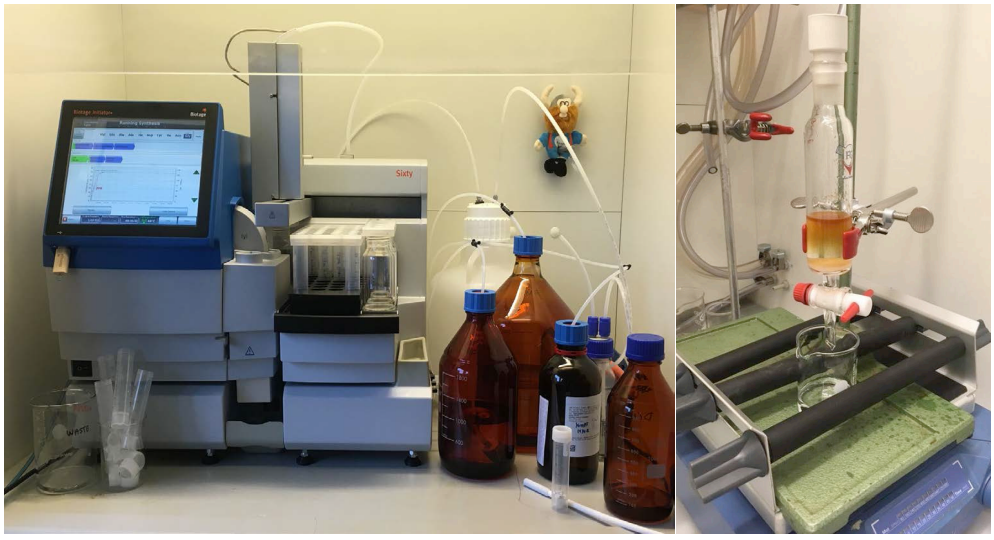


Figure: Left: solid-phase peptide synthesis performed on automatic peptide synthesizer (LOKE). Right: On-resin peptide cyclization.

P17: BioZEment 2.0: Metabolic modelling, reactive transport modelling, and microscale consolidation experiments in the production of bio-concrete

Author: Karlsen, Emil - NTNU

Introduction:
It is important to reduce human CO_2 emissions to mitigate climate change and its severe consequences. Conventional concrete production contributes approximately 5% to these emissions.

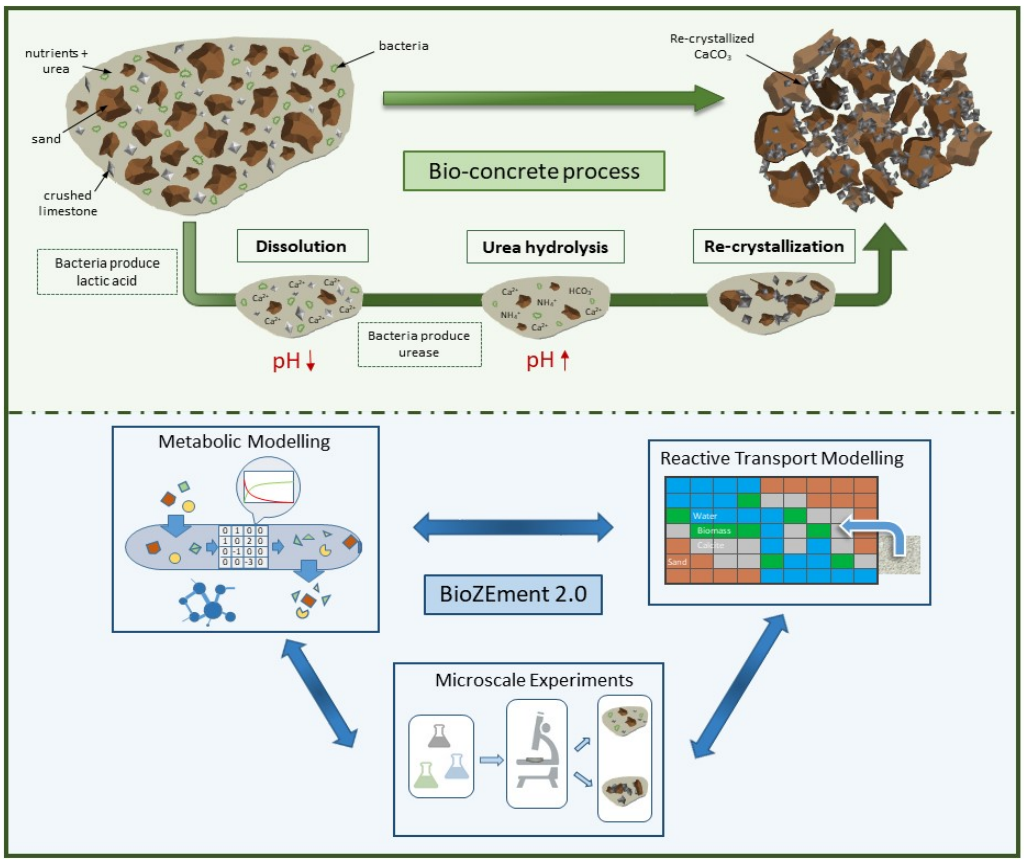
BioZEment 2.0 has the aim to develop a more sustainable, concrete-like material, using bio-catalyzed dissolution and re-precipitation of $CaCO_3$ (limestone). In a two-step process, we 1) use acid-producing bacteria to partly dissolve $CaCO_3$ grains mixed with sand, and 2) use ureolytic bacteria to produce urease, which hydrolyses urea to ammonia and carbonate ions, raising pH and re-precipitating $CaCO_3$ between the sand grains, forming a solid material. For a better understanding of the material formation process, it is crucial to have an in-depth understanding of the metabolism of the bacteria, the microscale formation process of the material, and their interplay.

Methods:
For the dissolution step, an acid-producing natural *Bacillus* isolate strain is used. A genome-scale metabolic model is developed to supplement experimental efforts in controlling the metabolic behavior of the bacteria. For the consolidation step, the bacterium *Sporosarcina pasteurii* is used as urease producer to induce a precipitation process in experiments. The resulting crystal growth is monitored with and without sand *in situ* and in real-time with optical microscopy and optical pH measurements.

The experimental results and bacterial modeling will be used as input for the simulations for a more targeted optimization of both the dissolution and consolidation processes. A reactive transport model with a chemical solver, using the lattice Boltzmann method, tracks geometric evolution, transport of nutrients and chemical species, and bacterial distribution over time on the pore-scale.

Results and discussion
A genome-scale draft model of the bacteria used for dissolution has been produced, currently containing 2024 reactions and 1086 metabolites. Inferred from genome annotation data and attributes of closely related species, this model will require extensive curation based on experiments. For monitoring of consolidation on the microscale, a sample cell has been developed that allows real-time monitoring in the presence of a granular medium with high spatial and temporal resolution. A reactive transport model using lattice Boltzmann method has been developed. Reactions added to the chemical solver and the model have been tested against benchmark examples, and bacteria have been placed in the model as point sources of reactants. Dimensionless analysis has been performed, and simulations can show how the system responds to changes in dimensionless numbers.

Figure:


nutrients + urea
bacteria
sand
crushed limestone
Re-crystallized $CaCO_3$
Bio-concrete process
Bacteria produce lactic acid
Dissolution
Urea hydrolysis
Re-crystallization
Bacteria produce urease
pH ↓
pH ↑
Metabolic Modelling
Reactive Transport Modelling
Water
Biomass
Calcite
Sand
BioZEment 2.0
Microscale Experiments

P18: Lab-on-a-chip platform for early detection of disease biomarkers

Author: Aksnes, Astrid - NTNU

Introduction

Early and reliable detection of diseases is desirable because it dramatically increases the chance for successful treatment. An increasing number of diseases can be detected at an early stage and monitored due to the presence of certain biomarker molecules in the fluids of the human body (blood, saliva, urine, etc.). Today detection of protein biomarkers is routinely based on immunoassays in centralized labs.

The ability to conduct diagnostic functions on a lab-on-a-chip (LOC) is of great interest. Obtaining analyses results quickly on-site combined with reduced costs and higher throughput of assays are driving forces for LOC technology. Small chip sizes facilitate low sample volumes, allowing better control of molecular interactions close to the sample surface. The quality of transducers, microfluidics and functionalization processes have improved over the last years. However, it has proved challenging to fabricate inexpensive LOC with low limit-of-detection (LOD) and highly reproducible results, particularly in complex biofluids.

Methods

Our goal is to address these challenges by developing a multiplexed LOC for detection of biomarkers with improved sensitivity and selectivity compared to state-of-the-art. Laboratory functions are combined on mm^2-sized silicon-on-insulator (SOI) chip including biophotonic sensor elements, microfluidic channels and readout circuits. Microfluidic channels guide the transport of fluids containing target biomarkers to the multiplexed photonic sensing elements. These sensitive photonic transducers can detect refractive index changes due to the capture of biomarkers by antibodies immobilized on the sensor surface. By modifying the surface functionalization of the sensing elements, different biomarkers can be detected.

As a proof-of-concept, the sensor is designed for detection of 3 distinct antigens: C-reactive protein, lipocalin 2 and tumor necrosis factor. The main challenge lies within their respective concentrations and LOD and different dynamic ranges for each analyte, varying from μg/ml to pg/ml.

Results and discussion

LOC photonic sensor and microfluidic prototypes have been fabricated and characterized. Fig. 1 shows a schematic of the LOC with microfluidics and grating in- and outcoupling, as well as a photo of one of the prototypes. Grating incoupling of light has been introduced to ease alignment and improve optical efficiency. Robust protocols have been established for silicon-on-insulator processing and chemical functionalization (Fig. 2). Numerous transducer designs have been fabricated including ring resonator (RR), Mach Zehnder interferometer (MZI) and photonic crystal (PC) resonators, examples are shown in Fig. 3. The individual transducer designs facilitate different LOD's and dynamic ranges for each analyte. Measurements of biomarker concentrations in the μg/ml to ng/ml range yield promising results. There is ongoing work on the microfluidics, functionalization and photonic

transducer design to improve the LOD to enable measurements of concentrations in the pg/ml range.

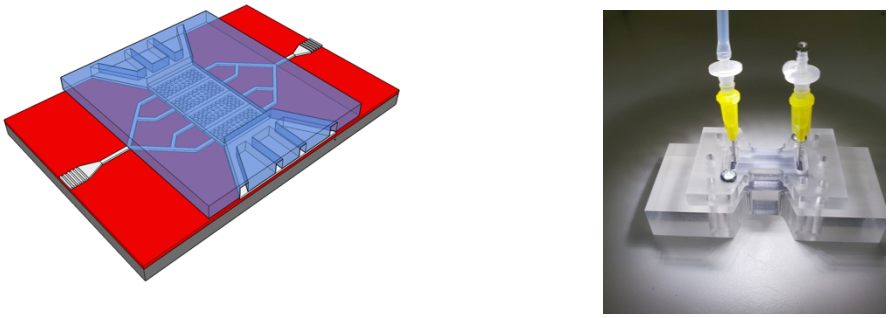


Figure 1: *Schematic of a 4-channel LOC with microfluidics and grating in-/outcoupling (left), and photo of a LOC prototype in a PMMA holder (right). Liquid is flowed through the system using syringes.*

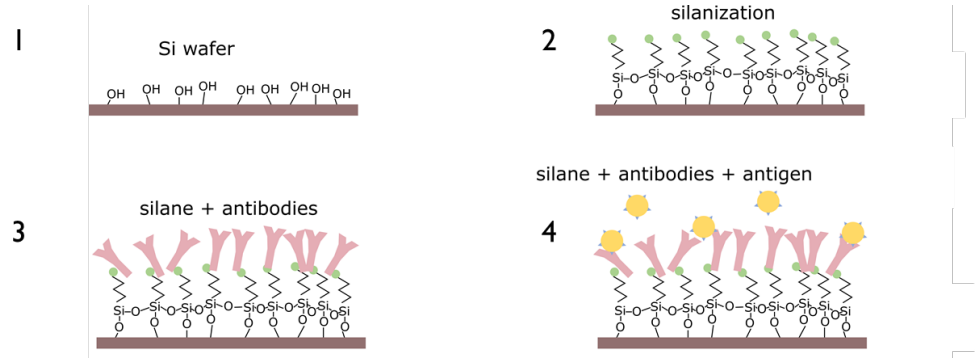


Figure 2: *Functionalization steps and capture of biomarker (antigen). The silicon chip is 1) cleaned with oxygen plasma, 2) silanized with APTES (NH2- terminated), 3) antibodies are attached locally to the silanized sensor surface using NHS/EDC-chemistry, and 4) the antigen is captured by the antibodies.*

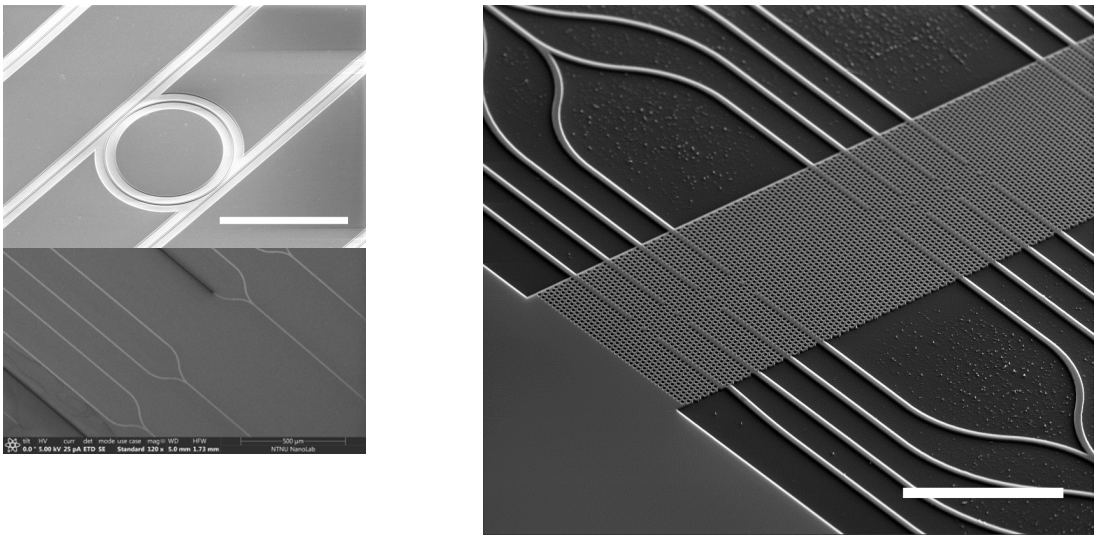


Figure 3: *Fabricated SOI transducer structures: ring resonator (top left), Mach Zehnder interferometer (bottom left) and multiplexed 8-channel photonic crystal transducer (right).*

P19: Anisotropic NMR for structural elucidation

Author: Rylandsholm, Fredrik Garnås - UiT The Arctic University of Norway

Introduction

When new natural products are discovered, an important part of the discovery is to find the structure of said natural product, including the absolute stereochemistry of all stereogenic centres. However, structural elucidation can sometimes be challenging; The structure of Commafric A (Fig. 1), a natural product extracted from the resin of the plant Commiphora africana, could not be fully elucidated by common methods. Therefore, the anisotropic methods residual dipolar coupling (RDC) and residual chemical shift anisotropy (RCSA) were utilised. RDC gives the relative orientation of the ^{1}H-^{13}C-bond in space, while RCSA gives the relative orientation of ^{13}C shielding tensors in space.

Methods

The compound is dissolved in $CDCl_3$ with a TMS reference. A polymer is then added at gradually increasing concentration, to partially hinder free tumbling in solution. RCSAs are collected through analysis of 1D ^{13}C spectra, while RDCs are collected through analysis of transformed 2D HSQC-IPAP spectra. The resulting dataset is then handled in MSpin and compared to computational values to find the best fitting conformer.

Result and discussion

For Commafric A, vibrational circular dichroism spectroscopy (VCD) and Nuclear Overhauser Effect (NOE) were used to find most stereocentra, but the differences between the two conformers of C-14 were not exclusively distinguishable. The combination of RDC, RCSA, computational methods, and the results from VCD and NOE, were used to find the correct conformer: *S*. The method could potentially be used for structural studies of peptides in bacterial membranes.

Figure

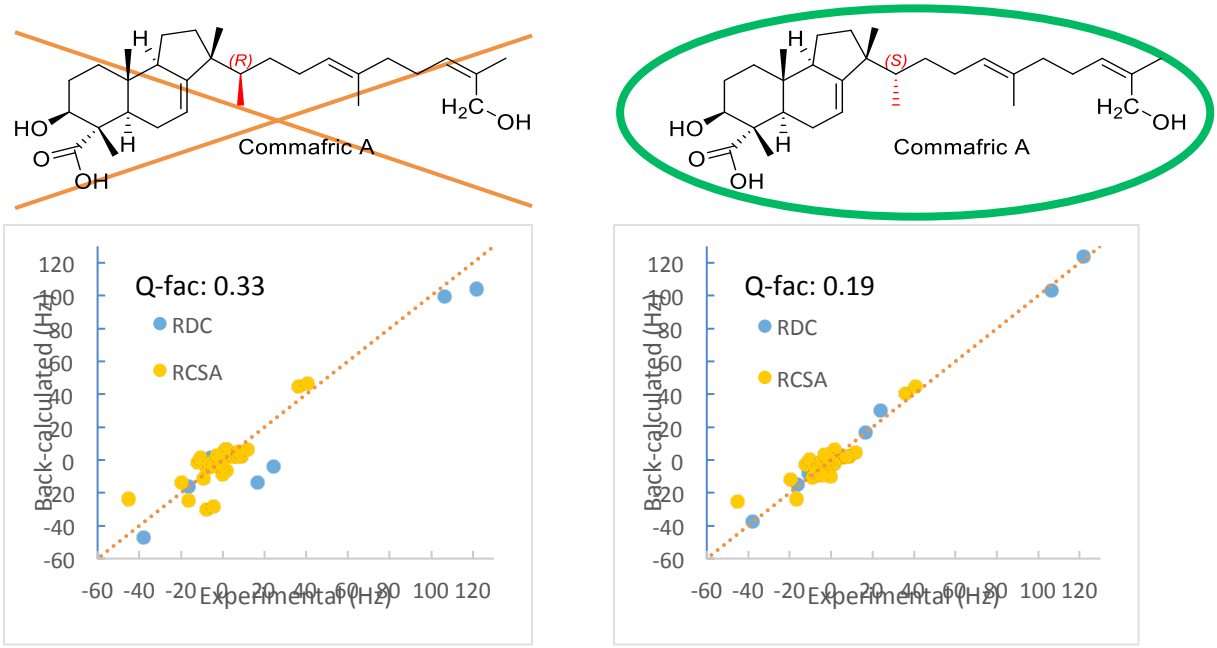


Fig. 1: Stereochemical differentiation between the two possible configurations of C-14 in Commafric A using RDC and RCSA, showing that S is the preferred conformer.

P20: Y-Net: A Deep Convolutional Neural Network to Polyp Detection

Author: Mohammed, Ahmed - NTNU i Gjøvik, IDI

AUTOMATED POLYP DETECTION

Introduction

Automated polyp detection techniques Tajbakhsh et al. (2016)Bernal et al. (2015)Angermann et al. (2016) can be used to mitigate the miss-rates and compliment colonoscopist in fast diagnosis. Our approach is different from other existing approaches in that we introduced a pre-trained encoder network that is augmented with untrained mirrored network and a decoder network that uses discriminative cost function to localize and detect polyps.

Our main contributions are: (1) A novel encoder-decoder network that uses a pretrained model for one of the encoders with mirrored untrained network and a decoder that is trained from scratch, making it more practical to train for polyp detection with limited amount of data. (2) A sum-skipconcatenation connection to a decoder network that allows re-use of pretrained encoder network weights. (3) Qualitative and quantitative experiments on MICCAI 2015 challenge on polyp detection Bernal et al. (2017) Bernal et al. (2015) Tajbakhsh et al. (2015) and comparison with state-of-the-art works Yu et al. (2017), show that our method outperforms in polyp detection with 7.3% F1-score and 13% recall improvement.

Methods

Our approach is illustrated in Fig. 1 and consists of two fully convolution encoder networks which are connected to a single decoder network. The main goal for having two encoders network is to address the performance loss due to domain-shift from pre-trained network (natural images) to testing (polyp data), leading to degradation in performance. Our approach focuses using the pretrained model features optimally by slow fine-tuning the pre-trained network and aggressive learning (i.e. higher learning rate) on the second encoder for a better generalization on the test set. The proposed model consists of two contracting paths on the left, i.e. encoders, and expanding path to the right, i.e. a decoder, that matches the input dimension. The decoder outputs a binary mask segmentation of the polyp on the last layer.

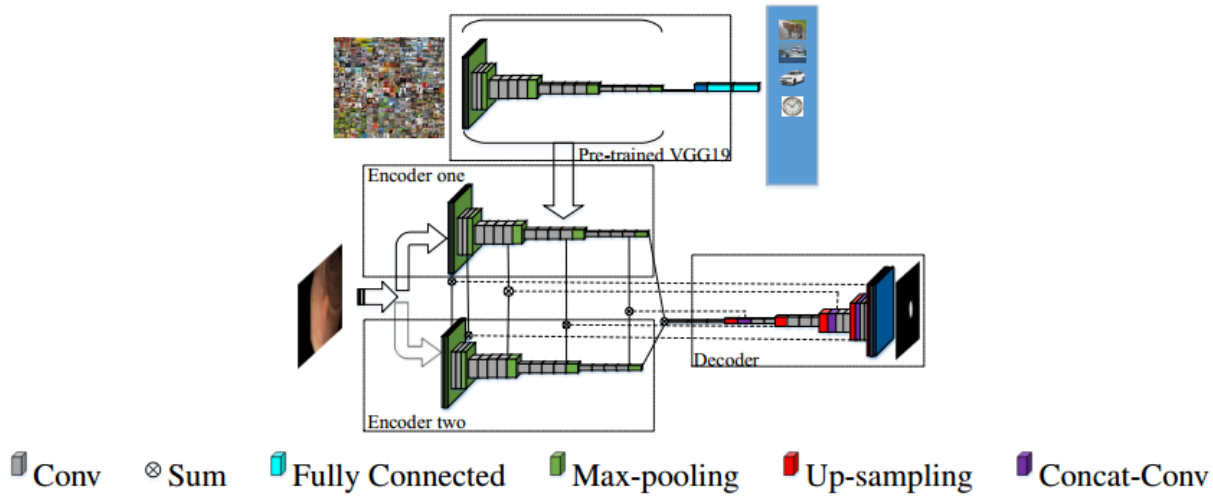


Figure 1: *Y-Net: proposed architecture. Given an input image, it is fed to both encoders. The weights of last convolution at each depth of the encoder are summed and concatenated to the same spatial depth of the decoder*

Result and discussion

Sample visual and quantitative results are shown in Fig. 2 and Table 1 respectively.

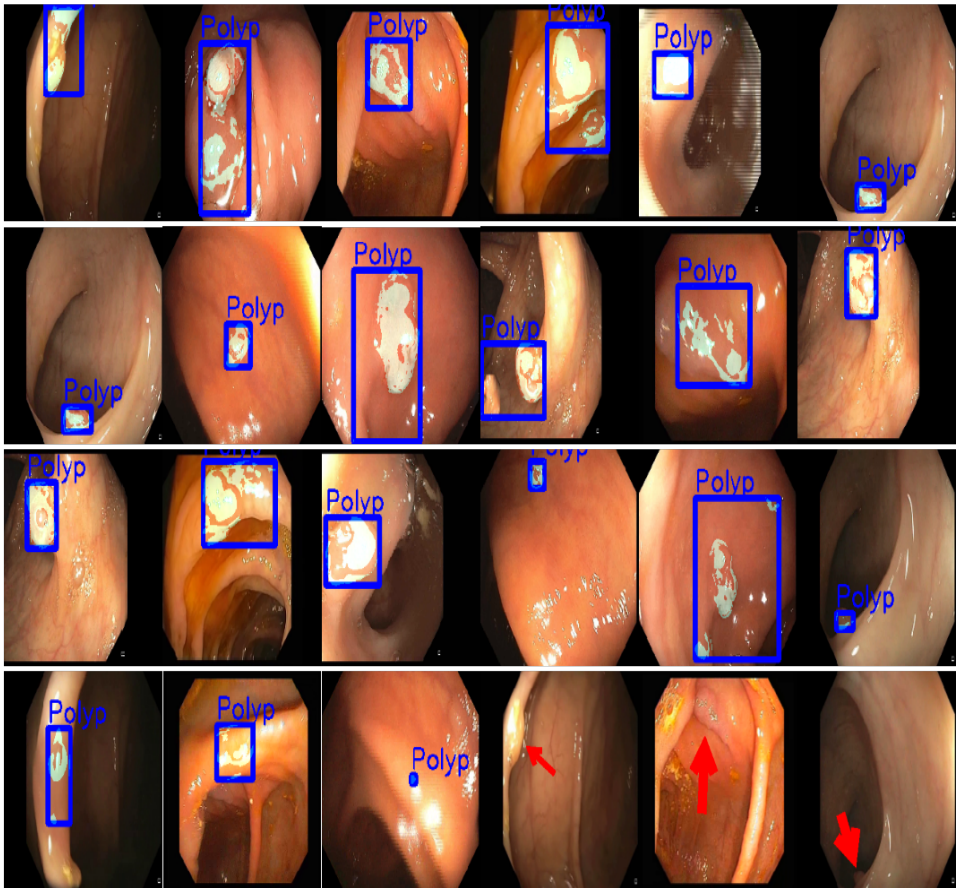
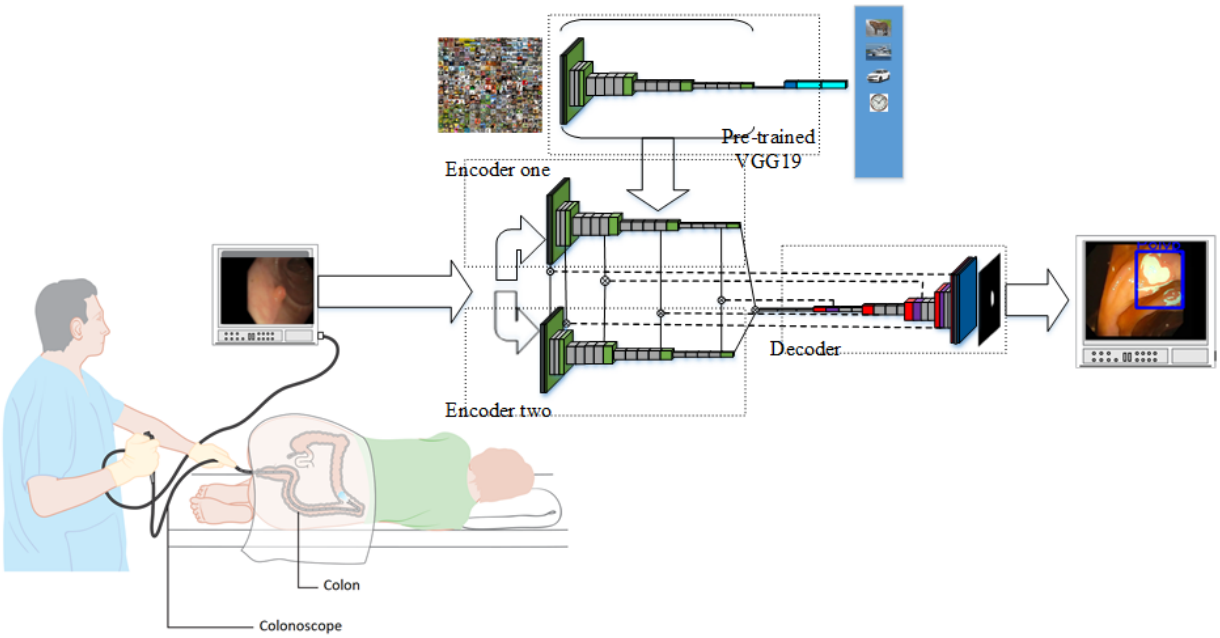


Figure 2: *Sample detection result on ASU-MAYO test dataset. The images are chosen to show variations of the polyp appearance and performance of our approach. The last row shows failure cases. The first three images show FP and the last three show FN.*

Table 1: *Comparison of different polyp detection methods on ASU-MAYO dataset. The result for PLS and OUS are published in Bernal et al. (2017).*

Method	TP	FP	FN	Prec[%]	Rec[%]	F1[%]	F2[%]
PLS	1594	10103	2719	13.6	36.9	19.9	27.5
CVC-CLINIC Bernal et al. (2015)	1578	3456	2735	31.3	36.6	33.8	35.4
OUS	2222	229	2091	90.6	51.5	65.7	56.4
ASU Tajbakhsh et al. (2016)	2636	184	1677	93.5	61.1	73.9	65.7
CUMED	3081	769	1232	80.0	71.4	75.5	73.0
Fusion Yu et al. (2017)	3062	414	1251	88.1	71.0	78.6	73.9
Y-Net(Ours)	3582	513	662	87.4	84.4	85.9	85.0

Y-NET: A DEEP CONVOLUTIONAL NEURAL NETWORK TO POLYP DETECTION



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Nima Tajbakhsh, Suryakanth R Gurudu, and Jianming Liang. Automatic polyp detection in colonoscopy videos using an ensemble of convolutional neural networks. In *Biomedical Imaging (ISBI), 2015 IEEE 12th International Symposium on*, pp. 79–83. IEEE, 2015.

Nima Tajbakhsh, Suryakanth R Gurudu, and Jianming Liang. Automated polyp detection in colonoscopy videos using shape and context information. *IEEE transactions on medical imaging*, 35(2):630–644, 2016.

Lequan Yu, Hao Chen, Qi Dou, Jing Qin, and Pheng Ann Heng. Integrating online and offline threedimensional deep learning for automated polyp detection in colonoscopy videos. *IEEE journal of biomedical and health informatics*, 21(1):65–75, 2017.

P21: Real Time Smoke Removal for Laparoscopic Surgery

Author: Wang, Congcong – NTNU

Introduction

In minimally invasive surgery, smoke generated by electrocautery and laser ablation deteriorates image quality severely. Figure 1 shows an example of laparoscopic surgery, a captured image with smoke and the corresponding one after smoke removal.

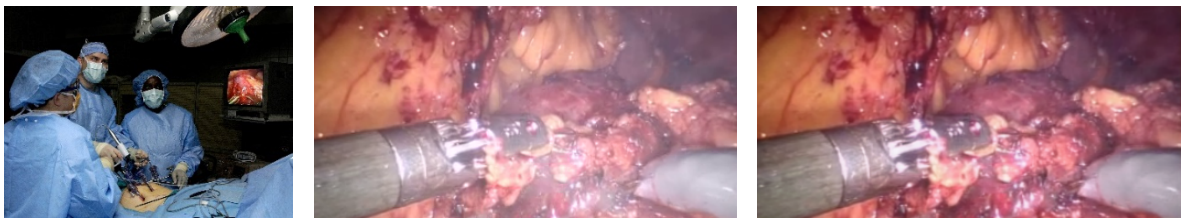

Figure 1. *Left*: An illustration of laparoscopic surgery. *Middle*: An example frame with smoke. *Right*: desmoked one by our method.

This creates discomfortable view for the surgeon which may increase surgical risk and degrades the performance of computer assisted surgery algorithms such as segmentation, reconstruction, tracking, etc. Therefore, real-time smoke removal is required to keep a clear field of view.

In this work, we propose a real-time smoke removal approach based on a Convolutional Neural Network (CNN). An encoding-decoding architecture with image pyramid decomposition input strategy is proposed. This is an end-to-end network which takes the smoke image and its pyramidal decomposition as inputs, and outputs a smoke free image directly without relying on any physical models or estimation of intermediate parameters. This design can be further embedded to deep learning based follow-up image guided surgery processes such as segmentation and tracking tasks easily.

Methods

1. Image Pyramid Decomposition

The original smoke image is decomposed into a N levels pyramid by the following strategy as shown in Figure 2, with $N = 5$ in this paper. For level n of the pyramid, the image is decomposed into a low frequency base layer and a high frequency detail layer by using guided filter as a low pass filter:

$$D_n(X) = O_n(X) - B_n(X), \tag{0.1}$$

where X is the original smoke image, n indicates the level of the pyramid with $n = 1,\ldots,N$. For decomposition level n, $D_n(X)$ is the detail layer and $B_n(X)$ is the base layer defined as $B_n(X) = guided(O_n(X))$, where $guided$ means guided filtering, $O_n(X)$ is the down-sampled image from $B_{n-1}(X)$, where $O_1(X) = X$, $B_1(X) = X$, a nearest-neighbor interpolation is applied for down-sampling.

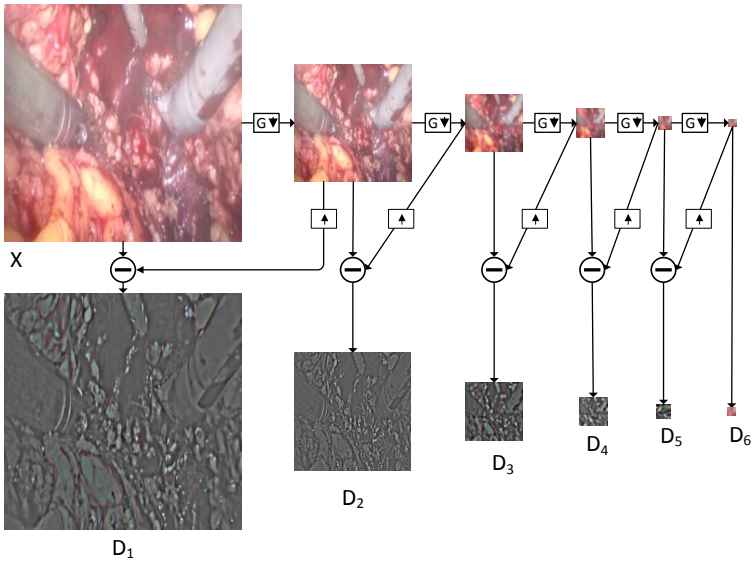

Figure 2. Image Pyramid Decomposition

2. Network Structure

An encoding-decoding network is adopted in this work and the network structure is shown in Figure 3. The encoder of our proposed architecture is similar to the encoding network of U-net, which includes five down-sampling (max pooling) operations and some '*Convolution (CONV)→(BatchNorm) BN→ReLU*' blocks.

The down-sampling operations cause spatial information loss, therefore, in addition to the original degraded image, the decomposition of the original images are concatenated to the corresponding levels of the encoder as shown in Figure 1. The original smoke image is input to level 1 of the network, detail layers D_2, D_3, D_4, D_5 are concatenated to levels 2, 3, 4, 5 of the encoder separately. This encoding structure is inspired by the Laplacian Pyramid and its application in the multi-scale fusion dehazing approaches. This design can compensate for the information loss and improve the convergence rate.

The decoder of the network consists of 5 up sampling operations, some '*Conv→BN→ReLU*' blocks. They are stacked as illustrated in Figure 3.

Besides, skip-connections between the encoder and decoder are introduced for information sharing from encoder to decoder.

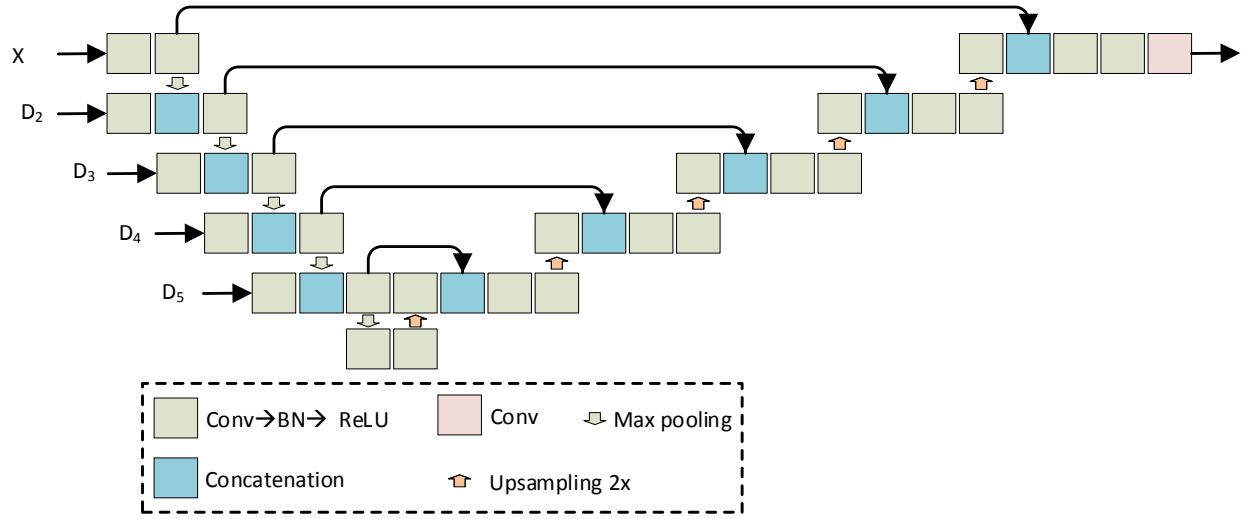

Figure 3. Network Structure

Result and discussion:

Training Data:

Manually select 7553 smoke free images from public dataset[1], synthesize smoke images by Blender and Adobe Photoshop with three smoke density: low, medium and high.

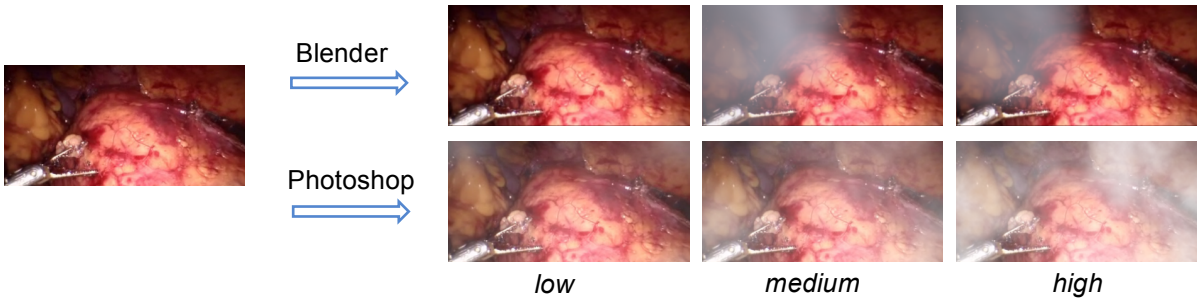

Figure 4. Examples of created synthetic smoke images.

Results:
Some visual results are shown in Figure 5 and 6.

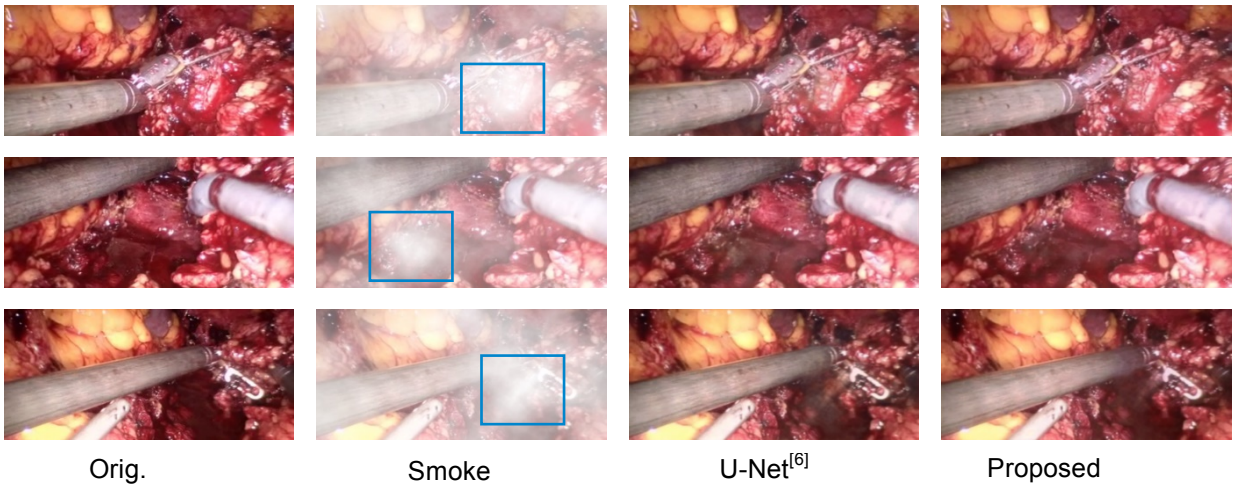

Figure 5. Subjective results of synthetic dataset

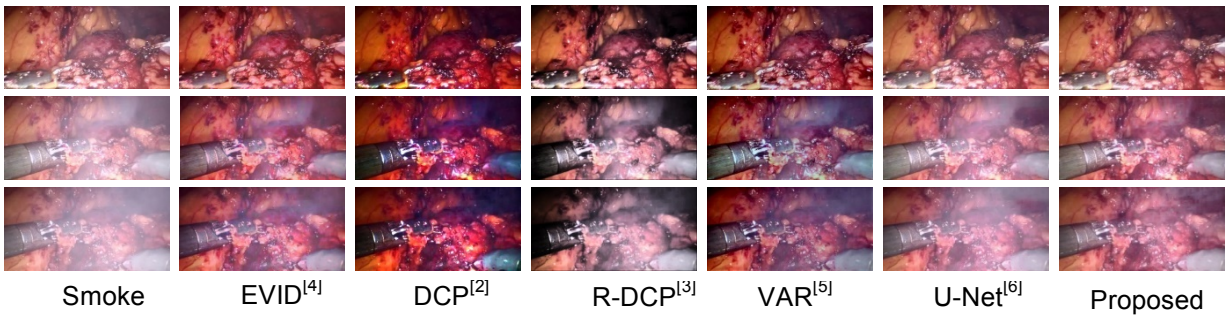

Figure 6. Subjective results of real smoke images

Discussion:
The proposed method reaches very good results on synthized images, but the performance degrades on real dense smoke images. Therefore, simulating more realistic training dataset to improve the results is necessary.

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P22: Metabolomics and fluxomics - essential approaches in optimization of bioprocesses

Author: Stafsnes, Marit - NTNU

Introduction
Metabolomics approach combined with fluxomics, enable accurate tracking of changes in the distribution of low molecular weight compounds in biochemical pathways, simultaneously allowing for utilization of those relevant information in the improvement of biotechnological processes. This poster will present our quantitative metabolite profiling work flow using as example results from the DLN project AurOmega. This project focus on understanding the mechanisms behind lipid accumulation and DHA production in thraustochytrids (see own poster).

The strain *Aurantiochytrium* sp. T66 cultivated and sampled under controlled laboratory conditions in bench-top bioreactors is used as a model organism. Levels of intracellular metabolites have been monitored in the different growth stages and reveals significant information about the actual state of examined cells. We have generated background data for evaluation of stress-responses and genetic perturbations in our research project. Initial data from batch cultivations on defined media grown with nitrogen limitation will be presented.

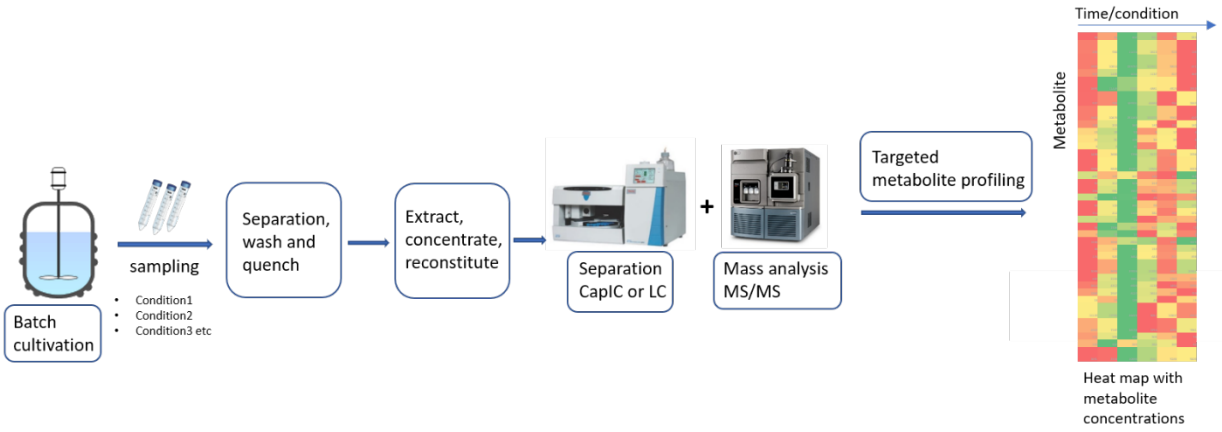
Methods
Batch fermentations in 1,5 L mineral medium with nitrogen limitation and glucose as the carbon source were applied. 13C-labeled glucose was used in fluxomics studies. The fermentations were carried out at 28 °C and the pH kept constant at 7.0. DO was kept at 20%. To obtain a metabolic “snapshot” the samples were immediately added ice cold mineral media followed by a quick centrifugation step (20 s). The amount of biomass per sampling was in the range 10 – 40 OD units. In this project it was crucial to limit the amount of biomass to ensure proper washing and quenching of the cells. The cells were disrupted with three freeze-thaw cycles (liquid N_2/water bath at 0°C), thereafter concentrated by lyophilization and reconstituted in MQ water.
Targeted quantitative mass spectrometry (MS) methods combined with a separation technique (LC, IC) covering the primary metabolite groups (amino acids, organic acids, sugar phosphates and other phosphorylated metabolites, complete nucleotide and nucleoside phosphate pools) have been used.

Result and discussion
In the initial stage of the project a completely defined mineral media for cultivation of *Aurantiochytrium* sp. T66 was composed. This enables accurate carbon balances and is essential in fluxomics when 13C glucose is utilized. The sampling protocol was optimized for this strain with respect to time consumption and reproducibility of results.

Sampling in the three different life stages in *Aurantiochytrium* sp. T66 (exponential, transition/N-depletion and lipid accumulation phase) during batch cultivations reveals high

variation in intracellular metabolite pool. In particular, at the onset of nitrogen exhaustion: citric acid accumulation, increased content of glycerol-3-P and decreased content of AMP.

For 13C fluxomics, minifermenters enabling cultivations with volumes as low as 0,1 L was designed to reduce the cost of cultivations. MS-based isotopic analysis was established on the CapIC-MS/MS platform for measurement of the ^{13}C-incorporation into metabolites.



P23: Industrial microbiology – 'omics methods and modelling as tools for process optimization

Author: Ertesvåg, Helga - NTNU

Introduction
Industrial scale bioprocesses using microorganisms as catalysts, or cell factories, have two product-independent aims: To maximise the production rates and to maximise the product yield on the carbon source. This implies that rate limiting steps in the synthesis must be identified and eliminated, and that as much as possible of the carbon source should be directed towards the wanted product.
To obtain this control, we need to understand the metabolic processes in the cells and the regulation of these. This knowledge needs to be integrated and transformed to a tool that can be used to identify targets for optimisation of the production strain by genetic engineering, as well as the conditions of the production process.
In the Digital Life project "AurOmega", the aim is to improve the microbial production of the essential n3 fatty acid DHA, for replacement of fish oil in salmon feed. The production organism, a thraustochytrid, store DHA as part of a triacylglycerol oil.

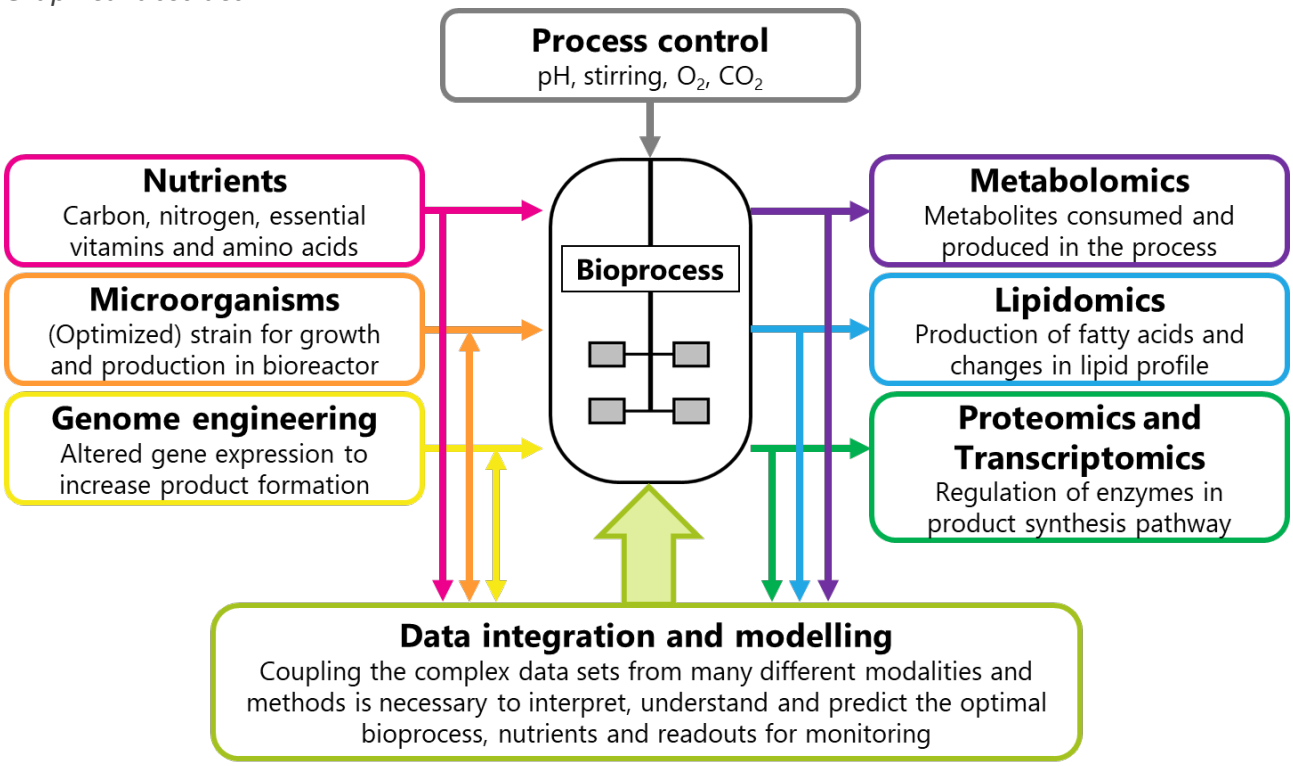
Methods
The project utilises a range of 'omics methods, as well as bioprocess engineering and mathematical modelling.
Bioreactor cultivations with control of pH and dissolved oxygen, and online monitoring of O_2-uptake and CO_2-emissions.
Transcript levels are studied by RNA-Seq and by ddPCR/quantitative PCR.
Shotgun proteomics, label-free or TMT-labelling, and targeted proteomics, with data analyses based on in-house, sequenced genomes and commercial tools.
Metabolomics: Targeted quantitative MS methods for the different metabolite groups have been established.
Fluxomics: ^{13}C-labelled glucose was used in the cultivation medium.
Lipidome, using supercritical fluid chromatography with tandem mass spectrometry.
Metabolic model: To computationally test experimental strategies through Flux Balance Analysis, a genome-scale metabolic model was built by applying the RAVEN toolbox to a model of a related strain, followed by manual curation.

Result and discussion
A draft genome sequence of our strain *Aurantiochytrium* sp. T66 was used to build a metabolic model containing 2093 reactions with 1191 associated genes.
Standardized protocols for the different analytical methods have been established. As part of this, a set of reference fermentations has been performed. Currently we are able to identify and quantify most primary metabolite groups, and a methodology for lipid profiling and quantification of lipid classes have been established.

Hypotheses that can explain observed changes in fatty acid profiles and lipid accumulation rates are supported by transcriptome and proteome data. Metabolome data have shown

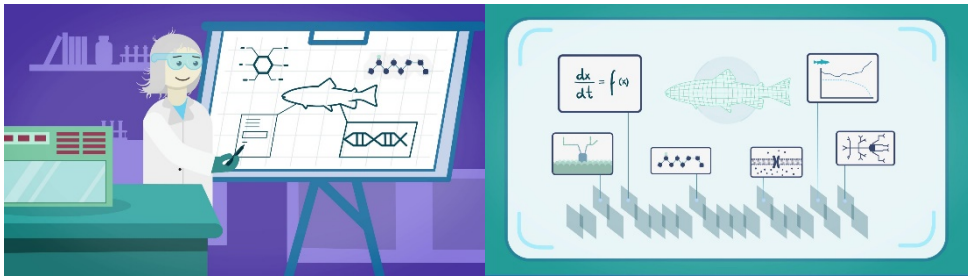
changes in some core metabolites when changing from growth to lipid accumulation, which support the hypothetical mechanism for initiation of lipid accumulation.
The expected project outcome will be an improved process that may include improved strains. Moreover, the expertise on the various methods will be directly transferable to other projects.

Graphical abstract .



P24: Towards a shared, pre-competitive knowledge base of mathematical models and data on salmon biology

Author: Vik, Jon Olav - Norwegian University of Life Sciences



Introduction
Salmon farming in Norway generates billions of euros annually but faces challenges regarding sustainability, animal welfare, disease, climate change and product quality. Addressing these challenges requires integration of the vast amounts of data and biological knowledge available. Mathematical modelling is key, transforming biological knowledge into numbers and equations so we can deduce new insights, reveal knowledge gaps, and evaluate possible remedies with the help of computer simulations. This needs to be a joint effort involving industry, academia and funding bodies, taking advantage of best practices from systems biology, information management and software development. A pre-competitive, shared knowledge base in the form of mathematical models linked to big data will enable continuous integration of knowledge and faster response to new challenges.

Methods
An initial Digital Life grant spearheads this initiative, addressing how novel sustainable feedstuffs get transformed into meat through salmon's metabolic reaction network. Data and model resources are made findable, accessible, interoperable and reusable by labeling their variables using terms from suitable *ontologies*, domain-specific vocabularies (e.g. gene names, labels of cellular compartments) that also describe the relationships between terms, enabling machine "reasoning" over the database. Datasets and models are furthermore linked in an Investigation-Study-Assay structure providing context regarding purpose, methods, and what related datasets exist. Industry partners provide know-how and relevant use cases. The first workshop with other industry companies was held in June 2019, seeking consensus and moral commitment to a shared, pre-competitive knowledge base of salmon biology.

Result and discussion
The industry workshop established a working group comprising industry, academia, the Research Council of Norway, the Norwegian Seafood Research Fund, and innovation facilitators. The working group is tasked with drafting a whitepaper describing the rationale for data sharing and reuse, identifying pre-competitive purposes of mutual interest to the participants, new business models, and possible organizations of the collaboration. A key consideration is combining short-term gain while realizing the long-term potential. Research

output so far includes a draft metabolic model for salmon, contribution to a metabolic model testing software, and systems analyses of gene expression remodeling from freshwater to seawater and its interaction with diet. Lessons and best practices from this work will be broadly applicable in digital production biology.

P25: Modelling salmon metabolism: Best practices for development and quality assurance

Author: Rotnes, Filip - NMBU

Introduction
Salmon aquaculture generates billions of euros annually, but faces problems of sustainability. Novel, sustainable feedstuffs are being trialed, but there is a need for a mathematical-biological framework to integrate data and understanding of the effects of novel feeds on salmon physiology. We develop the SALARECON model of salmon's metabolic reaction network, computationally studying the effects of potential feed mixtures. We adopt best practices for test-driven development, virtual experiments to assay metabolic capabilities, revision control, and FAIR data and model management, enabling fast, collaborative, reliable development.

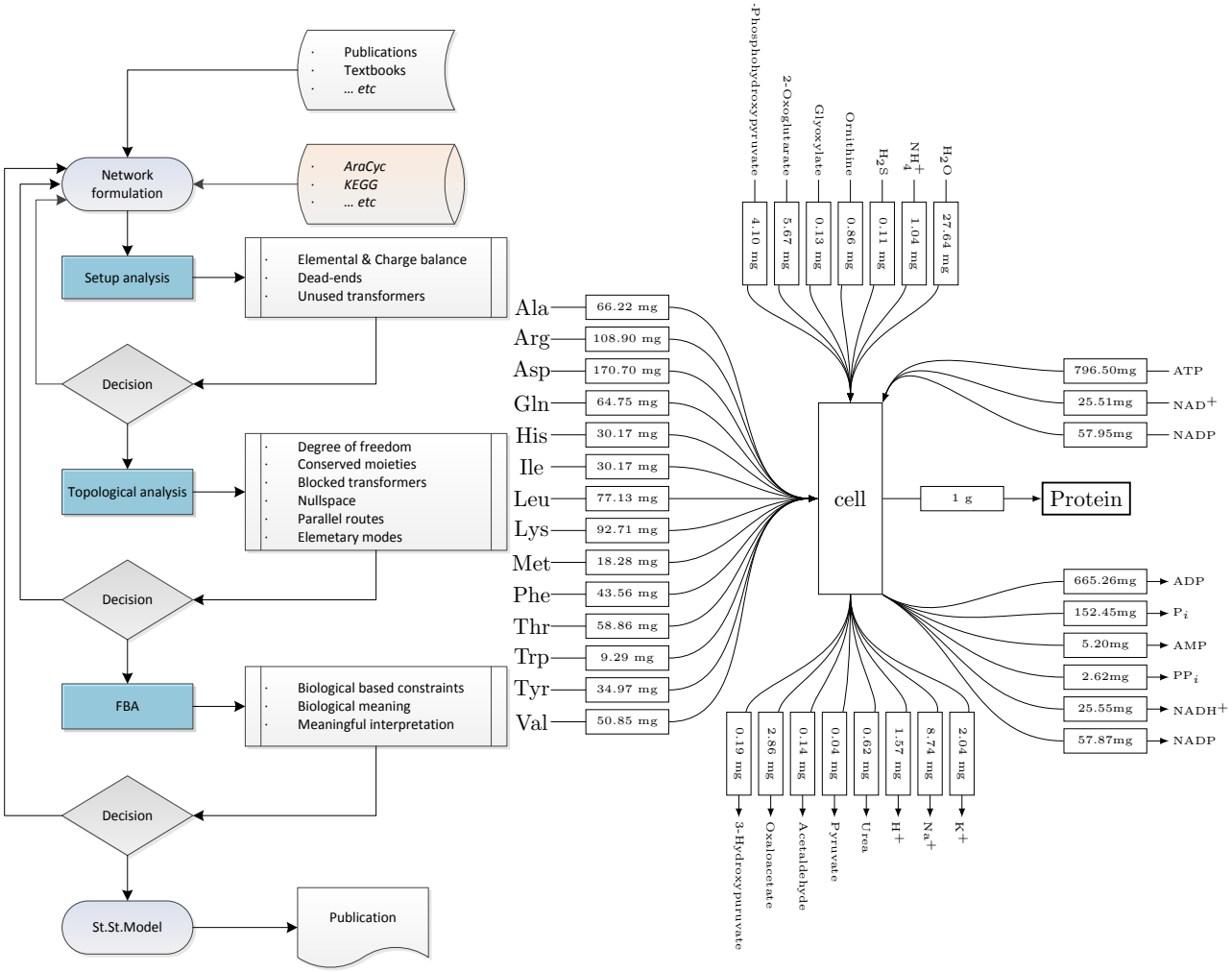
Documentation is a fundamental part of development of genome-scale models, as well as a means of making the models available in parallel with development as soon as possible. Using tools like Git, developers can document upgrades and improvements, and collaborate to fix potential weaknesses. Git also enables users to give feedback alongside development to ensure user-guided model augmentation.

Methods
The model is encoded in SBML3FBC format with genes, proteins and reactions annotated using standard ontologies and analysed using the Cobra toolbox for Python. The Memote software for metabolic model testing computes various measures of the model's metabolic capabilities, completeness of annotation, and systemic properties for every revision, tracked using the Git source control system. Model performance is plotted over time, documenting progress and detecting any emerging bugs. We simulate growth using flux balance analysis and predict biomass yield. Flux variance analysis allows us to simulate and test the feasibility of optimal growth conditions for the salmon along with variations in the range in input-compounds, such as vitamins and amino-acids from food.

Result and discussion
Memote tests metabolic map improvements over time with respect to various aspects. For instance, tests for SALARECON indicate a reduction of dead-end metabolites over time, while the metabolic network of the model has expanded.

Downstream applications of metabolic models including gene expression and proteomics data, which efficiently may integrate data generated by a variety of institutions and persons and enable detailed analysis of metabolic events.

SALARECON is a genome scale metabolic model enabling organism-level simulation of growth of Atlantic Salmon. Adopting best practices like version control and test suite construction have greatly contributed to ensure model quality and confidence and will continue to fuel validation processes in the future.


Publications
Textbooks
... etc
AraCyc
KEGG
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Network formulation
Setup analysis
Elemental & Charge balance
Dead-ends
Unused transformers
Decision
Topological analysis
Degree of freedom
Conserved moieties
Blocked transformers
Nullspace
Parallel routes
Elemetary modes
Decision
FBA
Biological based constraints
Biological meaning
Meaningful interpretation
Decision
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Publication
3-Phosphohydroxypyruvate 4.10 mg
2-Oxoglutarate 5.67 mg
Glyoxylate 0.13 mg
Ornithine 0.86 mg
H2S 0.11 mg
NH4+ 1.04 mg
H2O 27.64 mg
Ala 66.22 mg
Arg 108.90 mg
Asp 170.70 mg
Gln 64.75 mg
His 30.17 mg
Ile 30.17 mg
Leu 77.13 mg
Lys 92.71 mg
Met 18.28 mg
Phe 43.56 mg
Thr 58.86 mg
Trp 9.29 mg
Tyr 34.97 mg
Val 50.85 mg
cell
1 g
Protein
796.50mg ATP
25.51mg NAD+
57.95mg NADP
665.26mg ADP
152.45mg Pi
5.20mg AMP
2.62mg PPi
25.55mg NADH+
57.87mg NADP
0.19 mg 3-Hydroxypyruvate
2.86 mg Oxaloacetate
0.14 mg Acetaldehyde
0.04 mg Pyruvate
0.62 mg Urea
1.57 mg H+
8.74 mg Na+
2.04 mg K+

P26: Development of the aortic valve calcification treatment

Author: Zabirnyk, Arsenii - University of Oslo

Introduction
Calcific aortic valve disease is the most common valve disease in the Western world and it is the third leading cause of cardiovascular disease. This disease usually develops with the age and since the world has an ageing population, its burden on patients and society will increase. Currently the usual treatment option is open heart surgery with aortic valve replacement, although catheter-based techniques are being introduced. However, the heart valve prostheses being implanted have a set of complications. The aim of the project is to develop pharmacological treatment, which can inhibit calcification and prevent aortic valve stenosis.

Methods
We employed our group's expertise in the cellular and molecular mechanisms of aortic valve calcification and we have developed a high-processing in vitro model of the disease using human valve interstitial cells (VIC) which are known to be crucial for calcification. VICs in culture are regarded as a standard in vitro model of heart valve calcification. Healthy and calcified aortic valves are obtained from heart transplant recipients and patients undergoing aortic valve replacement due to calcific valve disease, respectively. VIC are isolated and stored in biobank until used. VIC are cultured in basic growth medium. Calcification is induced in the cell cultures by changing to osteogenic medium three weeks. After complete differentiation, calcification is spectrophotomterically measured. To investigate possible inhibitors different concentrations of the possible inhibitors are added to the cultures.

Result and discussion
After the primary screen and hit validation we have found six compounds which were able to stop the development of aortic valve calcification in a dose-dependent manner in the cell model. The effect differed between valvular interstitial cells donor types: cells from healthy and calcified valves. Further hit validation and optimal regimen are being developed in parallell with additional screening. These findings may develop into a promising future pharmacological therapy to treat heart valve calcification.

P27: Supercritical fluid chromatography with mass spectrometry: A versatile tool for lipid profiling and lipid class quantitation

Author: Bartosova, Zdenka - NTNU, Trondheim

Introduction

Lipids have multiple roles and functions across species (e.g. energy storage, cell protection, division and signaling) and their analysis has had growing interest in recent decades. Lipids are large group of compounds with high variation in their structure and polarity, which is demanding for analytical techniques available. Currently, direct infusion mass spectrometry and liquid chromatography – mass spectrometry are two main analytical strategies used for characterization of a lipidome, but the reliable quantitation of multiple lipid classes still remains challenging. Recently, we have established a high-throughput methodology for lipid profiling utilizing supercritical fluid chromatography. Lipids (such as MG, DG, TG, CE, Cer, HexCer, PC, PE, LPE, LPC, PG, SM etc.) are separated based on their polarity, meaning that each individual class is eluted in a discrete zone which can be easily utilized for their quantitation.

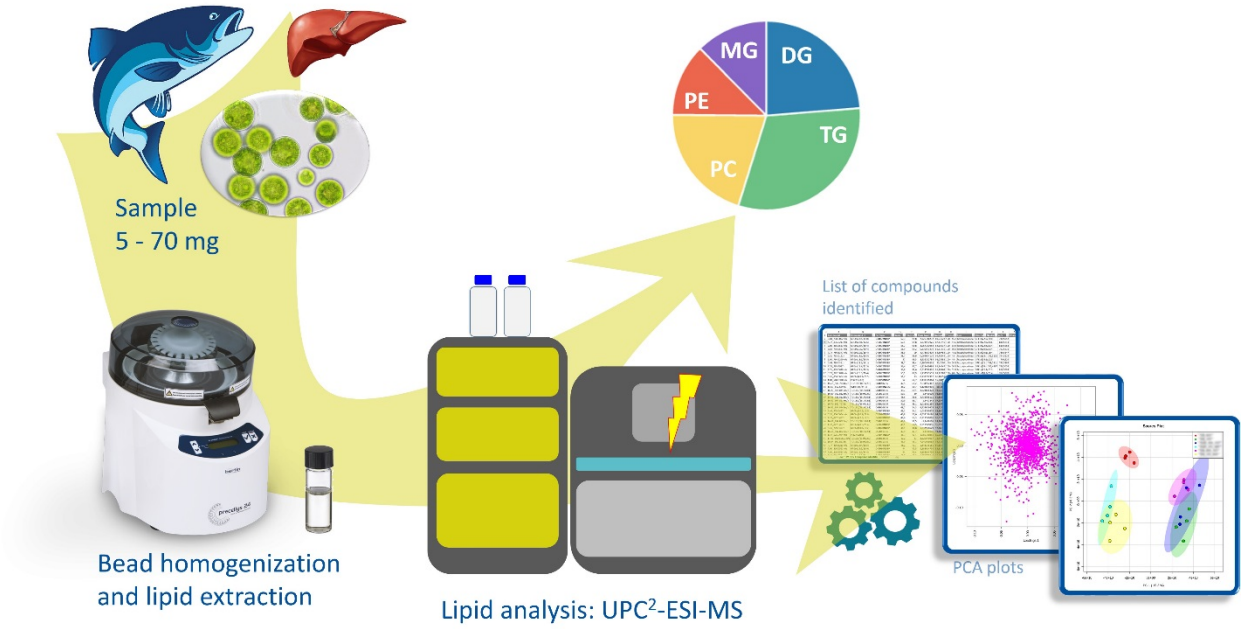
Methods

Suspended cells were extracted using a two phase system (water-methanol-chloroform) and a Precellys bead homogenizer. The extracts were subsequently analysed with supercritical fluid chromatograph (Acquity UPC^2, Waters) coupled to a Synapt HDMS QTof mass spectrometer (Waters) operated in MS^E. Raw non-targeted data were processed using Progenesis software with in-built LipidMaps and LipidBlast databases for compound identification. An in-house algorithm has been developed to extract and summarize integrated peaks of individual lipid species to obtain total abundances of individual lipid classes. Quantitation of lipid classes is based on single point internal standard method which provides results comparable with external calibration method.

Result and discussion

Presented method workflow is a versatile tool suitable for analysis of samples of various origin (e.g. tissue, bacterial cells, microalgae). Non-targeted lipidomic data have a great potential to reveal e.g. diet changes or lipid metabolism disorder when multivariate statistics is applied. Moreover, the application range of the method can be easily extended as the lipidomic data obtained can be utilized for lipid class quantitation.

We have applied our method for lipid profiling and quantitation of lipid classes in marine heterokonts thraustochytrids (*Aurantiochytrium* sp. T66). In agreement with literature the lipidomic data obtained confirm accumulation of triacyglycerols containing polyunsaturated fatty acyl chains, such as C22:6, C20:5 or C18:3. Furthermore, lipid extracts of T66 contain diacylglycerols, monoacylglycerols, phosphatidylcholines and phosphatidylethanolamines, however these lipids are less abundant and their total content is typically lower than 5% in accumulation phase.


MG
DG
PE
TG
PC
Sample
5 - 70 mg
List of compounds
identified
PCA plots
Bead homogenization
and lipid extraction
Lipid analysis: UPC²-ESI-MS

P28: Understanding interactions between membrane active molecules and biological membranes with NMR using nanodiscs as a membrane model system

Author: Rainsford, Philip - UiT

Introduction

Atomic-level descriptions of drug-target interactions are key to the rational structure-guided design of many pharmaceuticals. However, for antimicrobial peptides (AMPs), quantifiable Nuclear Magnetic Resonance (NMR) parameters are not easily obtained in simple lipid membrane models, which offer either high structural resolution or biological relevance. Further, the efficacy of some AMPs is such that these models are not robust enough to accommodate AMPs due to degradation. Nanodiscs[1] have been demonstrated to be stable membrane models that allow for high stoichiometric control, and are commonly used to study membrane proteins by NMR[2]. This project is seeking to reconcile the shortcomings of current NMR studies of membrane targeting middle-space molecules, with the strengths of nanodiscs, to allow for the extraction of biologically relevant structure parameters at a high resolution.

Methods

1) Nanodiscs are assembled as per literature procedures[1] by combining the desired lipid, solubilised in detergent, with the membrane scaffold protein. The subsequent removal of the detergent yields the nanodiscs, which are purified by gel filtration.
2) The AMP of choice is then introduced to the nanodisc in a molar ratio of 1-5 AMP : 1 nanodisc with 5 µL of D_2O.
3) The desired suite of spectra is then collected, including, but not limited to: t1/t2 relaxation, ^{2}D-NMR and, 1D and 2D spectra. Continued equal additions of AMP are made with data collected after each addition.

Result and discussion

AMP insertion can be confirmed by through-space AMP-lipid NMR signals that are present at higher concentrations of AMP. By monitoring the relaxation of different protons of the peptide in the presence of a paramagnetic salt, it is possible to identify which amino acid residues of the peptide insert into the disc, and which remain outside of the disc. Further parameters of interest are those that can help characterise the effects AMPs have on lipid bilayers, such as aggregation, perturbation, hydration and conformation within and without the membrane.

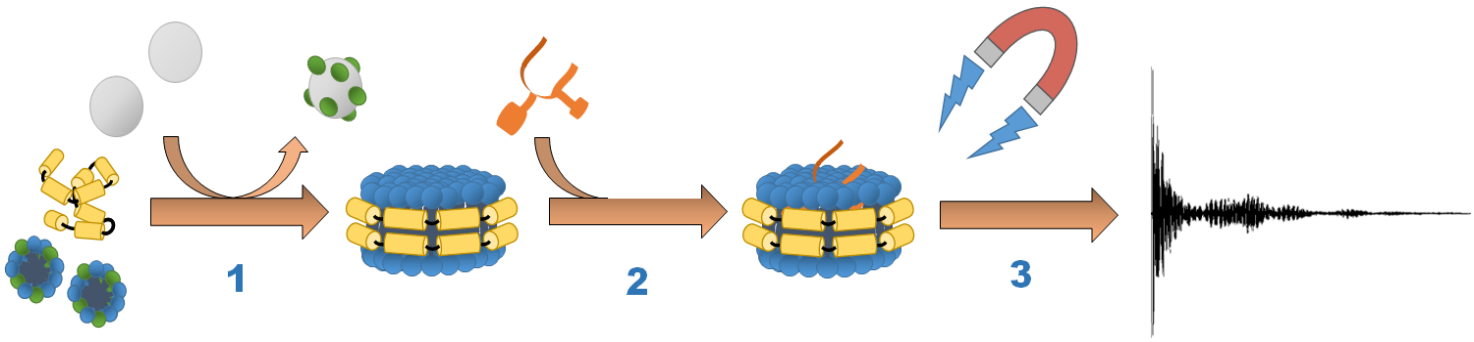


Figure: *General overview of the proposed workflow. 1) Preparation of empty nanodiscs. 2) Introduction of peptide of interest. 3) Collection of NMR data.*

[1] Bayburt, T. H., et al. (2002). "Self-Assembly of Discoidal Phospholipid Bilayer Nanoparticles with Membrane Scaffold Proteins." Nano Letters 2(8): 853-856.

[2] Nasr, M. L., et al. (2017). "Covalently circularized nanodiscs for studying membrane proteins and viral entry." Nature methods 14(1): 49-52.

P29: Prevalence of Hepatitis E Virus in Blood Donors in Islamabad, Pakistan

Author: Khattak, Jabar - International Islamic University, Islamabad

Introduction:
Hepatitis E Virus (HEV) is a single stranded, non-enveloped RNA virus belonging to the genus Hepevirus of the Hepeviridae family. This family comprises viruses which infect mammals, including humans, birds and fish. HEV has five genotypes: genotype-I (Asia, Africa), genotype-II (United States), genotype-III (Mexico), genotype-IV (China) and genotype-V (Europe). HEV1 and HEV2 infect only humans. These are endemic in developing/underdeveloped areas of Asia, Africa, Central and South America, where faecal-oral spread mostly occurs through contaminated water and causes outbreaks or sporadic cases. Clinical situation is mainly developed in young adults and is severe, associated with high mortality in pregnant women and patients with chronic liver disease. HEV-III and HEV-IV infect various domestic and wild mammals such as pig, wild boar, deer and rodents in addition to humans. HEV types infecting humans and animals in the same area are usually closely related phylogenetically, favoring zoonotic transmission. HEV-III is universal, while HEV-IV is predominant in Asia.

HEV is transmitted by eating contaminated, raw or undercooked meat, meat products and shellfish, and also by direct contact with already infected animals, causing autochthonous sporadic infections. Most of HEV infections are asymptomatic; clinical disease mostly affects middle aged and old men with underlying illness and immunosuppressed individuals. HEV can be transmitted by blood transfusion. Transfusion-transmitted HEV infection has been reported in several countries but its true frequency is probably underestimated because it is often asymptomatic and testing of blood donors is infrequent.

Results and discussion
The prevalence of HEV in blood donors is not studied in Pakistan until now. It is already reported that blood donors are a source of transmitting HEV and high prevalence of the virus is reported in developing and developed countries. We will collect samples from the blood banks of Islamabad oakistan registered with Islamabad blood Transfusion Authority and analyze for prevalence of HEV antibodies. Positive samples will be further processed for Polymerase Chain Reaction to confirm the presence of HEV RNA. Prevalence will be analyzed on the basis of final results by genomic analysis. As the hepatitis E is highly endemic in Pakistan, this study of prevalence of HEV in blood donors will help the policy makers to improve the standard of blood safety in the country

P30: Multiple kernel learning with structured Gaussian processes: an application to drug interaction prediction

Author: Rønneberg, Leiv - University of Oslo

Cancer drugs have the potential to be more effective when given in combination. When two or more drugs are combined, they may reinforce one another, producing a combined effect larger than could be expected had the drugs acted independently. Community efforts to develop predictive models for these combination effects, utilizing data from large-scale ex-vivo screening experiments, have highlighted the complex nature of drug response, in which nonlinear modelling and efficient use of prior biological knowledge are crucial for predictive performance. We propose a multiple kernel learning (MKL) approach, that allows the flexible integration of different data sources, learning convex combinations of kernels encoding different notions of similarity. In addition, kernels for drugs and cancer cell lines are combined using a Kronecker structure which, coupled with structured Gaussian processes (SGP), allow fast parameter learning and inference. In combination, this approach allows full uncertainty quantification of predictions, the inclusion of relevant biological knowledge, and opens the door for sequential optimisation in the design of drug combination experiments.

P31: Bio-Engineered Palladium Nanoparticles (BEDPAN)

Author: Joudeh, Nadeem - University of Oslo

Palladium nanoparticles (Pd NPs) are used in a wide range of electronic devices, energy cells, medical tools, and chemical industries. These NPs are currently produced by conventional chemical and physical methods that involve toxic and expensive chemical agents. However, the use of microorganisms to produce nanoparticles offers an environmentally friendly alternative.

One additional advantage for the biological production of Pd NPs is the ability to use genetic engineering to optimize the production process. Most importantly, this will allow us to fine-tune the physical properties of the NPs produced. In order to do so, the genes and pathways involved in this process should be revealed first.

For this matter, we have generated a transposon library and we have identified some genes and pathways. The aim of our project is to engineer bacterial cells to produce Pd nanoparticles of different sizes and shapes. These two features are important parameters that directly affect catalytic properties and how the particles can be utilized for various uses. The engineered bacteria will create new tailored materials for applications in medicine, chemical catalysis, and electronics.

P32: Analysis of proteomics data

Author: Brun, Morten - University of Bergen

The performance of different statistical and machine learning methods on proteomics expression data is presented. With data from codfish exposed in vivo to pollutants in eight different combinations.

P33: dCOD 1.0: DECODING THE SYSTEMS TOXICOLOGY OF ATLANTIC COD (*GADUS MORHUA*) – HIGHLIGHTS SO FAR

Authors: Anders Goksøyr1, Guttorm Alendal2, Libe Aranguren-Abadía1, Augustine Arukwe3, Nello Blaser2, Morten Brun2, Malin Celander4, Karina Dale1, Dorothy J. Dankel1, Nancy Denslow5, Marta Eide1, Shirin Fallahi2, Håvard Frøysa2, Fatemeh Ghavidel6, Siri Øfsthus Goksøyr1, Jed Goldstone7, Bjørn Einar Grøsvik8, Eileen Marie Hanna6, Ketil Hylland9, Inge Jonassen6, Essa Khan3, Rune Kleppe6, Roger Lille-Langøy1, Jan Ludvig Lyche10, Ian Mayer10, Mette Bjørge Müller10, Pål A. Olsvik8,11, Daniela Pampanin12, Cinta Porte13, Hans J. Skaug2, John Stegeman7, Zhanna Tairova9, Fekadu Yadetie1, Xiaokang Zhang6 & Odd André Karlsen1

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The Atlantic cod (Gadus morhua) is an important species in North Atlantic fisheries, as well as coastal and pelagic ecosystems. It is also a widely used indicator species in European monitoring programs. The goal of the dCod 1.0-project is to combine competencies in environmental toxicology, biology, bioinformatics and mathematics across the traditional department boundaries, to create a deeper understanding of the Atlantic cod's adaptations and reactions to stressors in the environment. Building on thorough studies and mapping of the cod genome and long research traditions on the physiology, toxicology and reproduction biology of cod, the dCod project will expand our knowledge with genomics based methods, with studies of how the cod genome responds under different environmental conditions.

In dCod 1.0 we have so far generated large amounts of experimental samples and data using *in silico (*defensome modelling and metabolic reconstruction*), in vitro* (luciferase reporter assays with cod nuclear receptors and aryl hydrocarbon receptors*), ex vivo* (precision-cut liver slices), aquaria and field studies, as data sets for bioinformatics analyses and mathematical models that can describe responses based on different scenarios. Overall, the ambition is to create a knowledge-base and a tool for environmental monitoring and risk assessment. Here we will highlight some results and experiences during the first years of the project involving lab and field studies, omics analyses, and transdisciplinary activities.

The dCod 1.0 project is funded under the Digital Life Norway initiative of the BIOTEK 2021 program, and the iCod 2.0 project under the ECOSYSTEM program of the Research Council of Norway (project no. 248840 & 244564).

P34: Chemical warfare in the aquatic environment; the chemical defensome networks of model fish species

Authors: **Marta Eide**[1], Xiaokang Zhang[2], Odd André Karlsen[1], Eileen M. Hanna[2], Jared V. Goldstone[3], John Stegeman[3], Inge Jonassen[2], Anders Goksøyr[1]

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Introduction:
The chemical defensome comprises an integrated network of gene families and pathways that together function to metabolize and eliminate harmful compounds [1]. It is critical for survival and highly conserved from invertebrates to fish and mammals. The chemical defense genes of Atlantic cod (Gadus morhua), a commercially and ecologically important species, are poorly studied. The object of this investigation is to assess the chemical defensome network of cod and compare it with the defensome networks of four other model fish species: zebrafish (Danio rerio), three-spined stickleback (Gasterosteus aculatus), Atlantic killifish (Fundulus heteroclitus) and Japanese medaka (Oryzias latipes).

Method:
To identify the genes related to the chemical defensome of the fish species, we performed hidden Markov model (HMM) searches using HMMER and defensome-representative Pfam profiles in the ENSEMBL assemblies. Suggested homologs for the retrieved protein sequences were found using reciprocal or one-way best hit BLAST searches against the well-annotated zebrafish proteome. Secondly, to ensure that we included any defensome genes not identified by us but annotated by ENSEMBL, we also searched gene names directly. The resulting lists of protein sequences were then refined to contain only chemical defensome related gene families and subfamilies according to previous definitions [1].

For Atlantic cod, the first approach was also performed using the peptide sequences from a new trinity assembly, providing better sequence coverage as it is based on the most recent cod genome assembly [2] and transcriptomics data.

Results:
The identified genes comprising the chemical defensome in the five model fish species was visualized in a network based on protein-protein interactions from the STRING database. Using available transcriptomics data, the tissue-specific expression of the genes was also assessed. A better knowledge of the genes and proteins involved in the chemical defensome networks will benefit toxicogenomic studies of the individual species, as well as the extrapolation of results from model animals to other species. In order to have a comprehensive model for environmental risk assessments, we also plan to incorporate the metabolic reconstruction of Atlantic cod in the future.

The study is part of dCod 1.0, part of Centre for Digital Life Norway (project no. 248840), and iCod 2.0 (project no.244564), funded by the Research Council of Norway and the University of Bergen. A trans-Atlantic mobility grant was also financed by the Ocean Outlook project/Bergen Marine Research Cluster.

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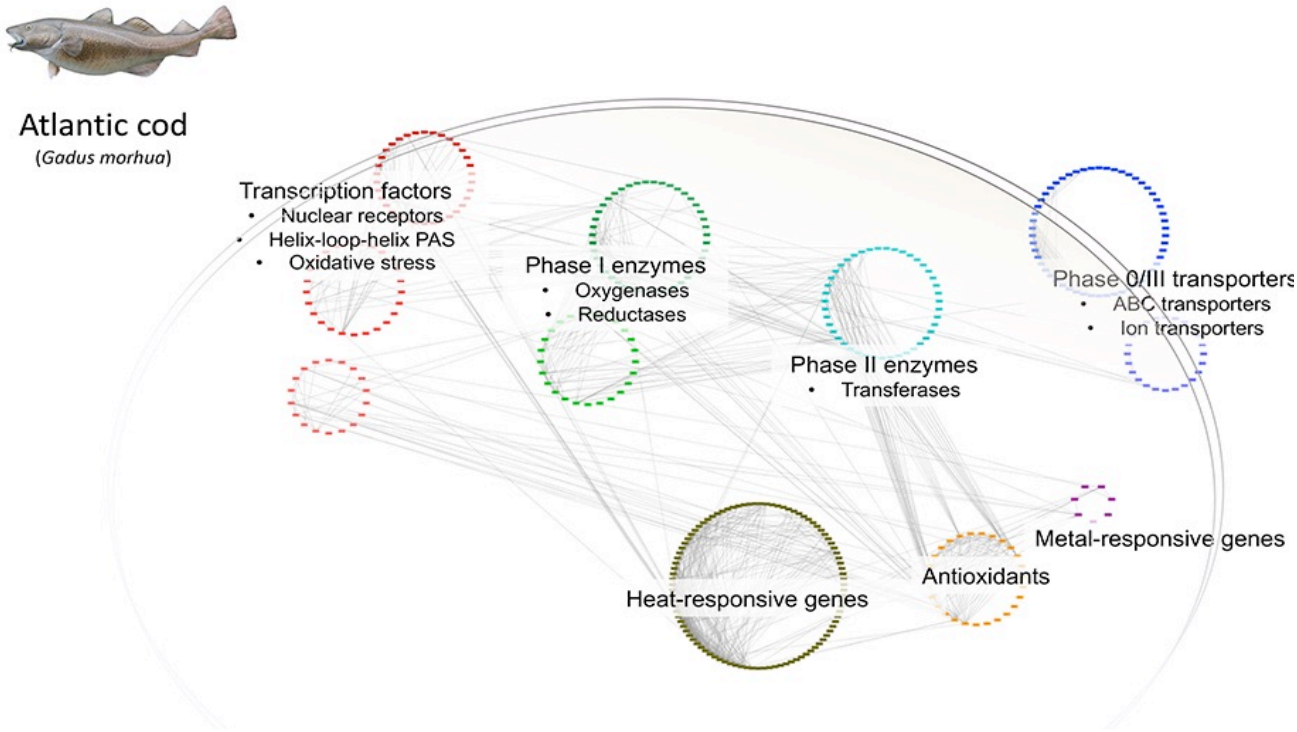

P35: Social Interaction in Research: Examining the Digital Life Norway project dCod1.0 with RRI methods

Authors: Elske Koelma[1,2] & Dorothy J. Dankel[2]

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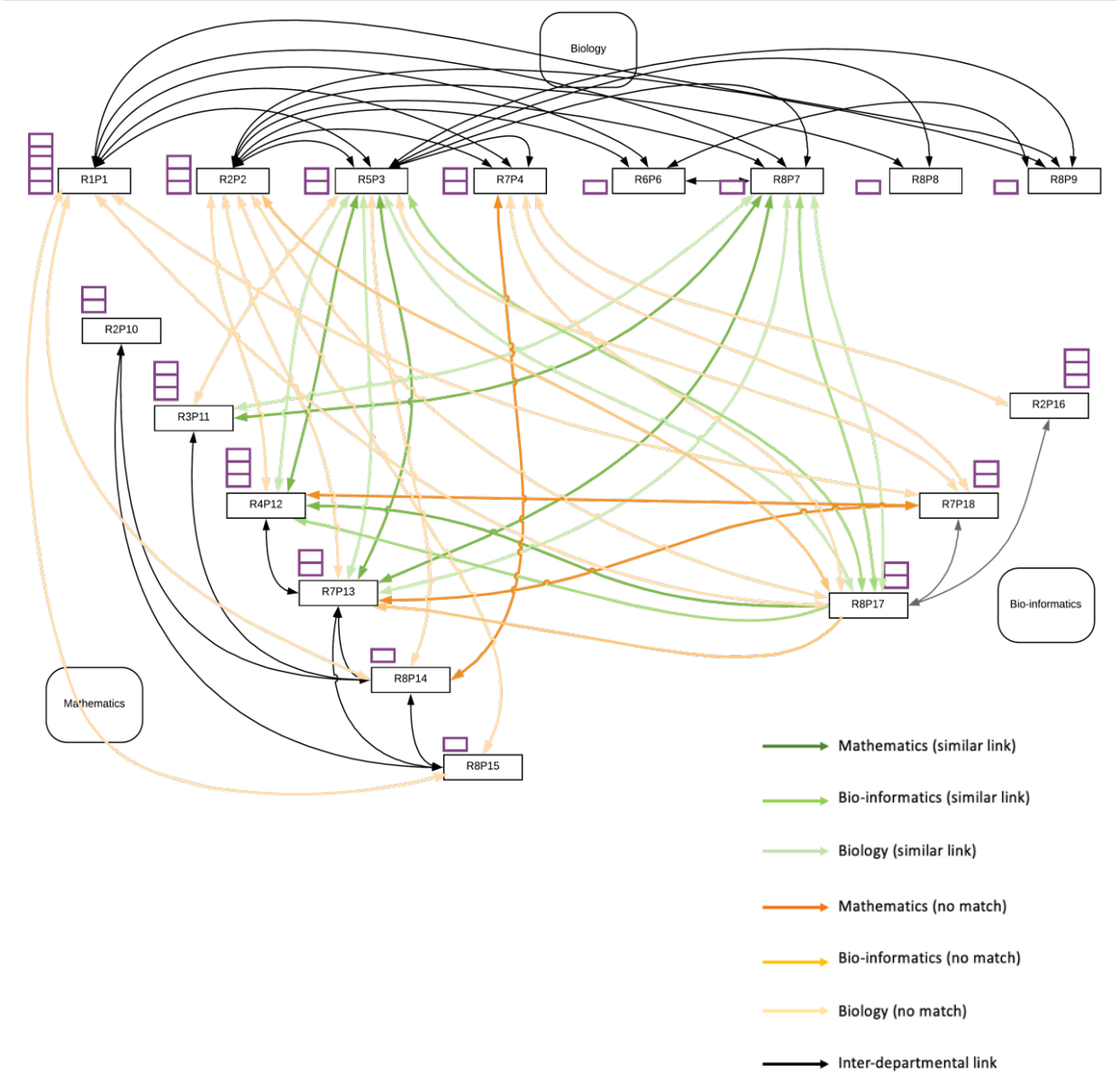
Poster Abstract: The ambition of DLN is inter- and transdisciplinary research and innovation that includes systems biology, mathematics, physics with social science and humanities embedded in the cross-cutting theme of "Responsible Research and Innovation" (RRI) The vision is that future innovations can be better realized through rational design and modeling based on a better systems understanding. But in order for these innovations to be socially robust, DLN has enforced a requirement for each project to include RRI perspectives for each project. But how can we "do" RRI? What is the added value of RRI in a DLN project?

This poster uses social science and humanities related methods in the frame of RRI to describe the interdisciplinary collaboration within the University of Bergen, Faculty of Mathematics and Natural Sciences within the project dCod 1.0. The methods conducted in the dCod 1.0 project group consisted of authority mapping sessions to determine concrete inter-departmental collaborations, a questionnaire to induce self-reflection, interviews and a final workshop where the project members discussed possible new collaborations to extend the current interdisciplinary collaboration. The "social" network analysis and authority mapping was particularly helpful for the consortium to visualize how they practice interdisciplinarity in practice (see figure below). The overall result of the RRI interventions is that they are perceived as helpful for the dCod consortium at the University of Bergen in regards to anticipation of future science needs and reflexivity of the current consortium's work.

Figure 5: The results of the network mapping in regards to data sharing among individuals in the UiB node of dCod1.0 in 3 departments: Biology, Mathematics and Bio-informatics. The horizontal rectangles represent people and the arrows represent data sharing between individuals. The small purple rectangles represent self-assessments of "authority"within the dCod1.0 project.



P36: RASflow: RNA-Seq Analysis Snakemake Workflow

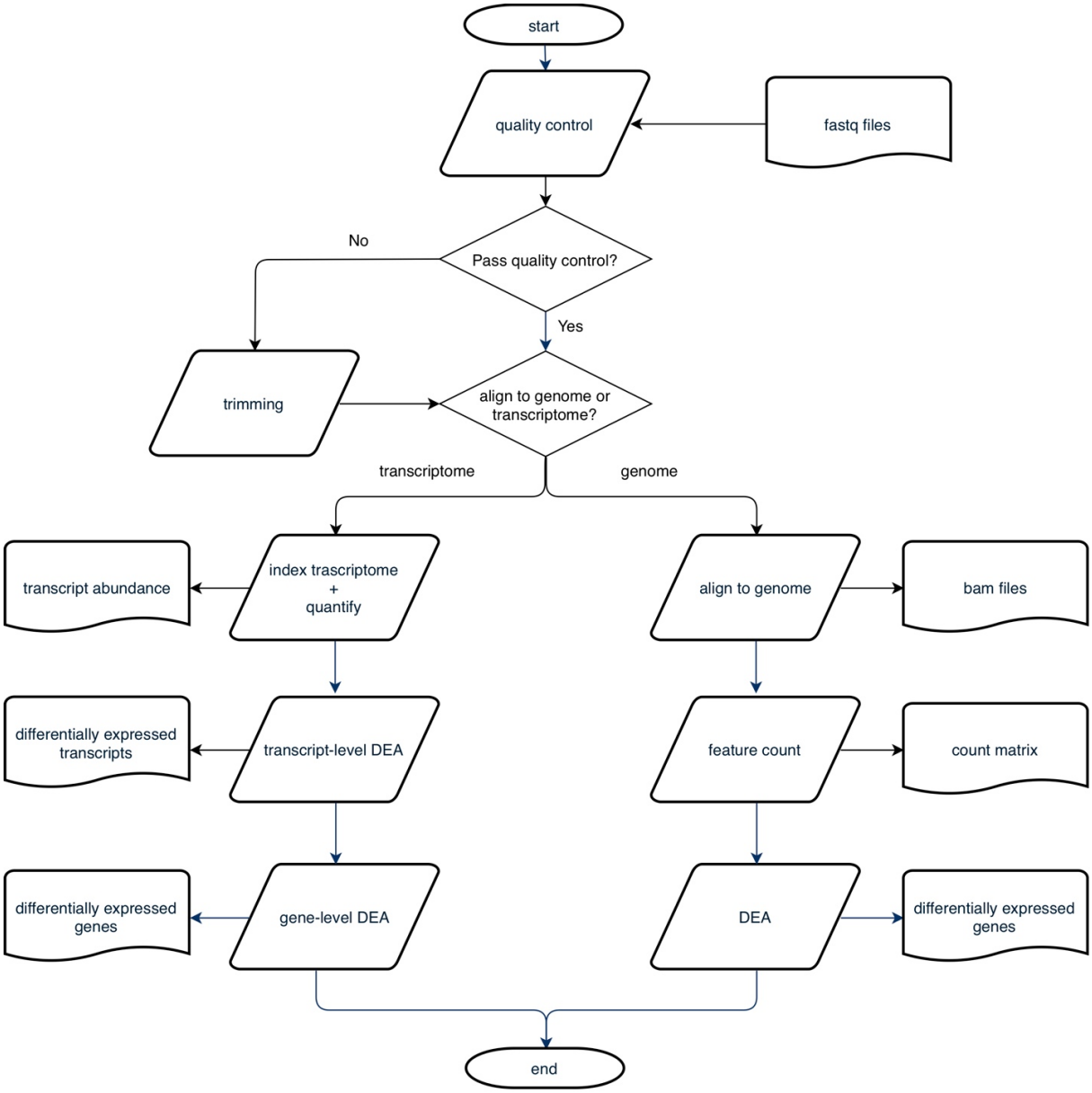
Authors**: Xiaokang Zhang**, Inge Jonassen

Computational Biology Unit, Department of Informatics, University of Bergen

Introduction:
With decreasing costs of DNA sequencing, increasing amounts of RNA-Seq data are being generated, having the potential to give novel insight into gene expression and regulation. Prior to analysis of gene expression, the data have to be processed through a number of steps resulting in a quantification of expression of each gene, or potentially splice form of a gene, in each of the analyzed samples. A number of workflows are available to help researchers perform these steps on their own data, or on public data to take advantage of novel software or reference data in data re-analysis. However, the workflows are usually limited to specific types of studies. We therefore aimed to develop a maximally general workflow, applicable to a wide range of data and analysis types and applicable for research on both model and non-model organisms. Furthermore, we aimed to make the workflow easily usable both for bioinformaticians and biologists with limited programming skills.

Results and discussion:
Utilizing the workflow management system Snakemake and the package management system Conda, we have developed a modular, flexible and user-friendly RNA-Seq analysis pipeline RASflow (RNA-Seq Analysis Snakemake Workflow). Utilizing Snakemake and Conda alleviates challenges with library dependencies and version conflicts and makes RASflow totally reproducible. To be applicable for a wide variety of applications, RASflow supports use of both genomic and transcriptomic assemblies. RASflow has a broad range of potential users: it can be applied by researchers interested in any organism and since it requires no programming skills, it can be used by researchers with different backgrounds. RASflow is an open source tool and source code as well as documentation, tutorials and example data sets can be found on GitHub: https://github.com/zhxiaokang/RNA-Seq-analysis

Graphical abstract



P37: Managing data through the FAIR principles: Collaborations for achieving open science

Author: Dale, Karina - University of Bergen

Data management can be defined as all activities necessary to make research data and related metadata discoverable, accessible and understandable today, tomorrow and well into the future. Data management should be performed according to the FAIR principles, where data is made **F**indable, **A**ccessible, **I**nteroperable and **R**eusable (Wilkinson et al., 2016).

In the dCod 1.0 project, expertise from toxicology, biology, bioinformatics, and mathematics converge to create mathematical models and biotechnological inventions, in order to provide better tools for environmental monitoring and risk assessment of the marine environment. To this aim, we have so far run eight large *in vivo* exposure studies, collecting numerous samples from 80-300 fish within each study. In addition, we have carried out a number of *ex vivo* exposures of liver slices, and *in vitro* ligand-receptor activation assays. These samples have been analysed to provide chemical data, transcriptomics, proteomics, and lipidomics, as well as targeted quantitative PCR and enzyme assays, generating a very large amount of data.

Thanks to the funding from Digital Life Norway for data management within the dCod 1.0 project, we have come a long way further in making our data follow the FAIR principles by:

- getting an overview of the available data within the project, and establishing a higher-level ISA structure appropriate for organising dCod 1.0 assets into SEEK inside the FAIRDOMHub (Wolstencroft et al., 2017)
- coursing of the hired data management assistant and people in the project that are central in the data assembling
- drafting templates and standardising attributes used for entering data into SEEK, and creating a step-by-step tutorial for project members
- started the process of entering the large amount of data sets (particularly biometric data), linking the different analyses of the specific fish/sample using established IDs
- generated open access to results data in publications, by providing a DOI for accessing all data linked to a publication (see e.g. Dale et al., 2019)

With funding from the Cross-project activities program of DLN, we have established contacts with other DLN projects such as DigiSal, Auromega and CCBIO to discuss and exchange best practices and experiences of data management. Through these collaborations, we hope to contribute to spreading the FAIR principles of data management.

References

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P38: Collaboration between Centre for Digital Life Norway, FAIRDOM and ELIXIR Norway to improve national resources for data management

Authors: **Fatemeh Z.Ghavidel**[(1)], Rune Kleppe[(1)], Kjell Petersen[(2)], Stuart Owen[(3)], Carole Goble[(3)], Inge Jonassen[(1,2)]

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With the significant advances in high-throughput omics technologies and consequently the impressive growth and availability of biological data, we may encounter new challenges for storage, sharing, and integration of the data. Data management (DM) is important for responsible research. It includes the storage, archiving and preservation of research data including required metadata. The goal of DM is to ensure that data is treated according to the so-called FAIR principles, that is, data should be made 'Findable, Accessible, Interoperable and Re-usable'. The active use of DM according to the FAIR principles is highly promoted and supported in the Centre for Digital Life Norway (DLN). Many of the DLN projects have been using the FAIRDOM/SEEK platform that is particularly adapted towards systems biology for managing and sharing scientific research datasets. Nationally, the Norwegian ELIXIR node develops and supports NeLS (Norwegian e-infrastructure for Life Sciences), a platform for omics data. DLN has supported an integration of the two platforms NeLS and SEEK performed by ELIXIR Norway and FAIRDOM.

The integrated of the platforms will make DM and long-term storage for Norwegian project leaders and collaborators easier, e.g. by solving challenges using voluminous omics data in system biology/medicine type projects while still supporting FAIR data and model management. Several Digital Life projects have participated in testing of the new versions of integration.

FAIR data management of non-sensitive data is now easier to do for Norwegian life scientists by combining features of NeLS and SEEK, allowing access to Norwegian infrastructure for storage, computing and archiving of research data as well as a rich toolbox for FAIR project data management.

P39: Mathematical modeling of regulatory interactions in fatty acid synthesis

Authors: **Shirin Fallahi**[1], Rune Kleppe[2], Anders Goksøyr[3], Guttorm Alendal[1]

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Introduction

Fatty acid synthesis (FAsyn) is a highly regulated process, adapting to the energy and lipid status of the organism. In animals, FAsyn mainly takes place in the cytosol and is specialized in tissues and cell types, with concurrent differences in the regulation. Cytosolic FAsyn rely on acetyl-CoA (A-CoA) derived from mitochondrial-exported citrate, with A-CoA carboxylation to malonyl-CoA (M-CoA) as the committing step that is feed-back regulated by CoA thioesters of fatty acids. M-CoA is the building block of fatty acid synthesis and fatty acid elongation in NADPH dependent reactions performed by multienzyme complexes. M-CoA also regulates mitochondrial fatty acid metabolism, by inhibiting the acyl-CoA transport of the mitochondrial Carnitine Palmitoyl-CoA Transporter 1 (CPT1). The pathway is thus regulated by allosteric interactions with metabolites within and outside the pathway, where the modulation varies in strength, depending on tissue expression of different enzyme isoforms that have distinct kinetic properties and responses to signaling pathways. We have used mathematical modeling to get better insight of the interplay and relationships between the regulatory interactions, the kinetic parameters of the enzymes involved and the pathway behavior.

Method

A liver model of FAsyn was constructed using mass action and Hill type kinetics, we constructed a model involving non-linear Ordinary Differential Equations. A synthesis pathway from A-CoA to Palmitoyl-CoA (P-CoA) is included with lumped reactions for the type I fatty acid synthase (FAS) and for consumption of P-CoA for triglyceride synthesis or elongation (Fig. 1). The pathway with regulatory interactions is shown by the diagram. The modeling and mathematical analysis were performed in Matlab (R2017b), using the Sobol sensitivity analysis approach available in *VARS-TOOL* [1] for sensitivity analysis and *fsolve* for steady state calculation. The model was imported into Copasi (version 4.16) for metabolic control analysis [2].

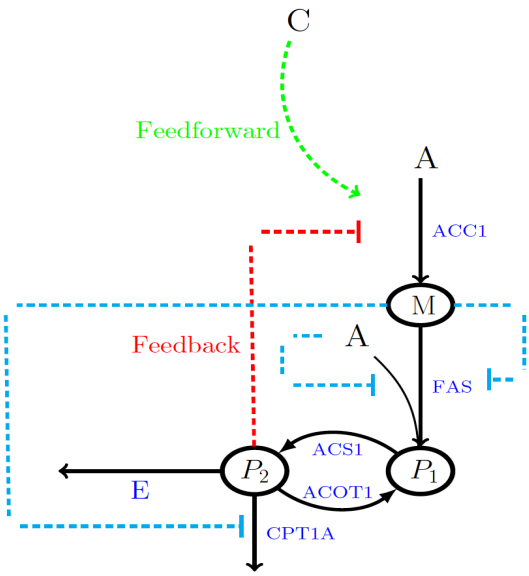
Results

A typical challenge for a modeler who develops a dynamic model of a biological system is lack of systematic measurement of kinetic parameters and characterization of steady states of the system. To face this challenge, we performed global sensitivity analysis known as Sobol sensitivity analysis to detect the most influential and non-influential parameters on the output of the system at the steady state level. Global sensitivity analysis guided us about which parameters were the most influential to reduce the total uncertainty in the model response. Then we performed the metabolic control analysis to identify the enzymes that had the most influence on the fluxes of the network at different regulatory states and enzyme expression levels. Furthermore, we have performed optimization studied of parameters and metabolite concentrations at the steady state phase, such that the flux to

mitochondrial beta-oxidation is minimized during FAsyn, without compromising the capacity to perform beta-oxidation at conditions where FAsyn is low.

Acknowledgements
This research was supported by the Research Council of Norway through grant 248840, dCod 1.0.

Fig 1: *A reaction scheme for fatty acid biosynthesis in liver with regulatory interactions. The metabolites are named; C (Citrate), A (Acetyl-CoA), M (Malonyl-CoA), P1 (Palmitic acid), and P2 (Palmitoyl-CoA). The regulated enzymes of each reaction are marked in dark blue. The reaction E represent a dummy enzyme that account for both microsomal elongation of P2 and esterification of P2 to triglycerides. The enzymes catalyzing the reactions are Acetyl-CoA Carboxylase 1 (ACC1, EC 6.4.1.2), Fatty acid synthase (FAS, EC 2.3.1.85), long-chain Acyl-CoA Synthetase (ACSL, EC 6.2.1.3), Acyl-CoA Thioesterase (ACOT, EC 3.1.2.2), Carnitine Palmitoyl-CoA Transferase 1A (CPT1A, EC 2.3.1.21).*



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P40: A draft metabolic reconstruction of Atlantic cod (*Gadus morhua*) liver

Authors: Eileen Marie Hanna[1], Xiaokang Zhang[1], Shirin Fallahi[2], Marta Eide[3], Fekadu Yadetie[3], Nello Blaser[2], Guttorm Alendal[2], Anders Goksøyr[3], Inge Jonassen[1]

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Introduction

The availability of genome sequences, annotations and the knowledge of the biochemistry underlying metabolic transformations helped generate metabolic network reconstructions of various organisms. Such networks include the form and function of genes in a target organism. When modeled using mathematical representations, a reconstruction can simulate the underlying genotype-phenotype relationships. Accordingly, genome-scale models (GEMs) can be used to predict the reactions of organisms to genetic and environmental variations. The interdisciplinary aspect and activities of the dCod project motivate our efforts to assemble existing knowledge on the Atlantic cod (*Gadus morhua*) into a metabolic reconstruction that can greatly support its evolving role as a model organism in environmental toxicology and other areas of biology.

Methods

Reaching a refined bottom-up metabolic reconstruction requires a long and strenuous process that typically consists of four main phases [1]. In the first stage, a draft reconstruction is generated from available sequences and annotations by retrieving reactions involving metabolic genes. Then, a refinement procedure follows to curate and balance the covered metabolic reactions. In the third phase, the refined reconstruction is converted to a constraint-based and condition-specific mathematical format. Finally, subsequent simulations and iterative validations can be performed using various datasets related to the target organism.

Results

We use the RAVEN (Reconstruction, Analysis and Visualization of Metabolic Networks) Toolbox 2 [2] which allows semi-automated reconstruction of GEMs, based on protein homology and using existing template GEMs and KEGG database. Focusing on the Atlantic cod liver, we use as template an existing curated human liver GEM that emphasizes lipid metabolism. Our preliminary draft model shows favorable retrieval of reactions from the template model to the draft Atlantic cod GEM, based on protein homology. The generated draft reconstruction is now subject to gap filling analysis and manual curation. Later refinement and simulation phases are as described above.

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P41: AHA: A simulation framework for modeling the evolution of cognitive architecture and decision-making

Authors: **Sergey Budaev**, Jarl Giske, Sigrunn Eliassen, Christian Jørgensen

Department of Biological Sciences, University of Bergen

Introduction

Decision-making is a fundamental problem for most organisms. It involves choosing one alternative among several available options. Except the most trivial cases decisions are made under uncertainty, the outcome is predicted. It depends both on the state of the decision-maker across many dimensions and numerous environmental factors. Any of them could change quickly. It is even not always clear what the notion of "the best" could really mean. Animals do not deliberate and think in terms of their future evolutionary success or weight myriads of conditional probabilities. However, even simple organisms have broad internal states like emotions and motivations. We argue that the the global organismic state—the subjective state of the "mind"—is fundamental for understanding the how and why of animal decision-making. This means that the organism is considered as an autonomous agent capable of goal-directed behavior, with the goals depending on its global state. The global organismic state integrates many diverse sources information, internal and external. It also withholds and ignores numerous sources of information deemed irrelevant. For example, the animal can be hungry or in state of fear. In state of strong fear information about a food item or a reproductive partner may be irrelevant to the current goal. Thus, emotions, motivations and the subjective "mind" provide the key components, proxies and the currency for decision making.

Methods

Behavioral ecology traditionally used analytical methodology by isolating a specific context and predicting what would be the "optimal" choice provided most relevant factors are known. Thus, optimal tactics and decision rules are inferred. Optimization here means maximizing some specific proxy for fitness, e.g. net energy intake. We follow the opposite path, by taking the perspective of the animal that is placed in uncertain environment and has limited information, not even granted "context." Essentially, it involves building a computational model of the whole organism—an artificial agent—that mechanistically implements behavioral and cognitive functions. Such an agent includes the genome, physiology, neurobehavioral architecture and interacts with the environment, e.g. finds food, avoids predators and responds to conspecifics. The genetic algorithm is then used to evolve solutions to genetic and cognitive/behavioral architecture in an individual-based model over numerous generations.

Results and discussion

We will describe the basic principles and software components of the framework which is thought as a digital laboratory. The main advantages of this approach is that evolution of the whole architecture rather than abstract tactics can be analyzed. The model results in a gene pool rather than specific abstract rule, so that genetic and phenotypic variation is accounted for. One example is the emergence of personality types with different sensory and emotional sensitivity. The software components are highly modular, follow the object-oriented approach, thoroughly documented and available as open source.

P42: Early Drug Discovery Using Experimental and Computational Approaches: Application to Antibiotics and COPD treatment

Authors: Fahimeh Khorsand[1], Ruth Brenk[1], Inari Kursula[1], Bengt Erik Haug[2], Åsmund Kaupang[2], Nathalie Reuter[2,3]

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Introduction

There is an increasing need for innovations in drug discovery in order to maintain the high health standards. While some drugs have been discovered through trial and error, modern drug discovery often starts from a validated biomolecular target for which modulators are sought using systematic strategies. Through this project, we aim at advancing two drug discovery projects addressing the need for (1) better drugs to treat Chronic Obstructive Pulmonary Disease (COPD) and (2) new antibiotics using expertise in organic synthesis, medicinal and computational chemistry, biochemistry, structural biology, mathematics, computer science, social sciences and law. In addition to the overall strategy, this poster presents the results of our work on finding a lead candidate for COPD treatment, and validating our target.

Methods

The small-molecule compounds are synthesized in-house and their activity assay on the targeted Neutrophil Serine Proteases (NSPs) are carried out using FRET substrates. The amounts of NSPs including PR3 and NE in patient and healthy samples were quantified by ELISA tests. The expression and purification of these enzymes will be carried out in insect cells to get the crystal structure of enzymes in complex with our promising small-molecule compounds.

Results and discussion

Around 70 compounds have been tested so far. And the best IC50 for PR3 is 800nM. We now have sufficient data to understand the Structure Activity Relationship (SAR) of the chosen scaffold. Analysis of bronchoalveolar lavage (BAL) fluid samples showed the elevated amounts of enzymes in patients vs controls and validated our drug targets. The experimental data provides input for computational drug affinity prediction and organic synthesis of new potential drugs.

Figure 1. Drug discovery and development pipeline featuring the steps of the hit-to-lead process and how far on the pipeline our two targets are: hit expansion of a promising series for Proteinase 3 while SAM-I RS is at the hit identification stage.