Nuoret Tutkijat/ Young Scientists

2023

Bioteknologian laitos
Department of Life Technologies

UNIVERSITY OF TURKU

NUORET TUTKIJAT / YOUNG SCIENTISTS 2023

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Akseli Jokela	Niko Peltonen
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Carla Vecenancio da Silva	Noora Tuominen
Eetu Maaranen	Nora Kullberg
Eetu Välimäki	Olli Kokko
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Ellinoora Koivula	Osama Mohamed
Elsa Xifre Pujol	Oskari Nääjärvi
Emilia Kaipainen	Pragya Karmacharya
Helea Junes	Rita Ojaniemi
Hille-Kaisa Leppänen	Saara Sahlman
Iida Payne	Saida Samadova
Iines Auravuo	Samantha Wadiyo
Jasmin Toukoniemi	Sanne Forsback
Josefiina Mäkinen	Shahrzad Bolouri
Juulia Allgaier	Shania Saini
Kaisa Leppä	Sowndharya Sankar Perumal
Katariina Kumpula	Suvi-Riitta Sulander
Laura Nikku	Sylvia Remes
Laura Tyni	Tiia Siivola
Lopamudra Chatterjee	Tuomas Rysä
Lotta Ristimäki	Tuulia Tuominen
Lucas Backström	Veera Luukkonen
Mafiur Rahman	Verneri Nissilä
Maiju Liimatainen	Xiangrong Fang
Malviina Nikola	Yasmin Alameldin
Mithila Ray	Zeynep Öztürk
Narges Moradi	

SEMINAR SESSIONS

Monday 27.03.2023 – Friday 31.03.2023
8:30 onwards
Pha1, Pharmacity, Itäinen
Pitkäkatu 4, 20520 Turku

POSTER SESSION

Wednesday 29.03.2023
12:15 – 13:45
Pha2, Pharmacity, Itäinen
Pitkäkatu 4, 20520 Turku

MAANANTAI / MONDAY 27.03.2023
8:30 – 10:00

·Opening· 8:30 **Prof. Jyrki Heino**

·Instructions· 8:35 **Esa Tyystjärvi**

Session I
Session host: Emil Aalto-Setälä

·1· 8:40

Sanne Forsback

Ihmisen pluripotenttien kantasolujen indusoiminen ja karakterisointi

·2· 9:00

Nora Kullberg

Molecular details of CIP2A-B56γ1 protein interaction

·3· 9:20

Lotta Ristimäki

Onkogeenisen kRAS proteiinin toiminnan estäminen

·4· 9:40

Ellinoora Koivula

Monosyyttiaktivaatiotesti pyrogeenien testausmenetelmänä

10:00 - 10:20 **TAUKO / BREAK**

MAANANTAI / MONDAY 27.03.2023
10:20 – 11:40

Session II
Session host: Anita Santana Sanchez

·5· 10:20

Tiia Siivola

Immobilization of genetically engineered *Chlamydomonas reinhardtii* to improve photobiotransformation of cyclohexanone to ε-caprolactone

·6· 10:40

Mithila Ray

Syanobakteerin *Synechocystis* sp. PCC 6803 sopeutuminen typen puutteeseen

·7· 11:00

Osama Mohamed

Construction and evaluation of translationally optimized engineered production pathways in the cyanobacterium *Synechocystis* sp. PCC6803

·8· 11:20

Anna Hämäläinen

In vitro studies of the activity of a cholesterol-forming enzyme

11:40 – 12:40 LOUNASTAUKO / LUNCH BREAK

MAANANTAI / MONDAY 27.03.2023
12:40 – 14:20

Session III
Session host: Liisa Pösö

·9· 12:40

Lopamudra Chatterjee

Molecular characterisation of PK-TF oncogenic gene fusions

·10· 13:00

Sowndharya Sankar Perumal

Hormonal regulation of the osteogenic signature in bone metastatic Castration-Resistant Prostate Cancer (CRPC)

·11· 13:20

Mafiur Rahman

Early detection of prostate cancer using Extracellular Vesicles (EVs) and NP-TRFIA

·12· 13:40

Hille-Kaisa Leppänen

Hypoksian vaikutus matriksin metalloproteinaasien ilmentymiseen okasolusyöpäsoluissa

14:00 – 14:20 TAUKO / BREAK

MAANANTAI / MONDAY 27.03.2023
14:20 – 15:40

Session IV
Session host: Erik Chovancek

·13· 14:20

Laura Nikku

Comparison of prime editing and base editing for generating the novel WRN^{R732P} mutation in breast cancer cells

·14· 14:40

Laura Tyni

Clever-1 interference to overcome resistance to immune checkpoint inhibitors

·15· 15:00

Pragya Karmacharya

Comparison of information dependent acquisition and sequential window acquisition of all theoretical mass spectra to characterize dynamics of faecal novel conjugated bile acids in relation to islet cell autoantibodies

·Alumni talk· 15:20

PhD student **Sofia Tyystjärvi**, Technical University Munich

TIISTAI / TUESDAY 28.03.2023
8:30 - 9:50

Session V
Session host: Jasmin Raita

·16· 8:30

Carla Vecenâncio da Silva

Sensory characteristics and phenolic profiles of the first generation interspecific hybrid strawberries

·17· 8:50

Noora Tuominen

Pastan rakenteelliset ja aistinvaraiset ominaisuudet teollisessa kuumennusprosessissa

·18· 9:10

Katariina Kumpula

Kasvipohjaisten elintarvikkeiden aminohappokoostumuksen tutkiminen

·19· 9:30

Tuomas Rysä

Alkoholittomien oluiden aistinvaraiset laatutekijät

9:50 - 10:10 TAUKO / BREAK

TIISTAI / TUESDAY 28.03.2023
10:10 – 10:50

Session VI
Session host: Yuqing Zhang

·20· 10:10

Nita Pokharel

Fatty acid composition of lipids in marine products, milk, and oils

·21· 10:30

Xiangrong Fang

Health effects and bioavailability of *n*−3 fatty acids

·22· 10:50

Saida Samadova

Encapsulated linseed oil ethyl esters and microalgae oil for food fortification: Fatty acid and volatile profiles

11:10 – 12:30 LOUNASTAUKO / LUNCH BREAK

TIISTAI / TUESDAY 28.03.2023
12:30 – 13:50

Session VII
Session host: Asiye Kazeroonian

·23· 12:30

Shania Saini

Development of lingonberry wines with a sensomics approach

·24· 12:50

Ada Okwum

Effect of yeast strains on the chemical composition of apple ciders from Finnish cultivars

·25· 13:10

Elsa Xifre Pujol

Impact of *Saccharomyces* and non-*Saccharomyces* yeast on fermented vinegar from Finnish cultivar apple peels

·26· 13:30

Zeynep Öztürk

Functional screening of beneficial strains and consortia to maximize healthy fermentation potential

13:50 – 14:10 TAUKO / BREAK

TIISTAI / TUESDAY 28.03.2023
14:10 – 15:30

Session VIII
Session host: Qizhu Zhao

·27· 14:10

Samantha Hedwig Wadiyo

Analysis of novel plant-based foods with ^{1}H Nuclear Magnetic Resonance spectroscopy

·28· 14:30

Onyinyechi Stella Kpaduwa

Sensory characterization of commercial plant-based meat analogues

·29· 14:50

Malviina Nikola

Fermentation of lupin with lactic acid bacteria using a bioreactor

·Alumni talk· 15:10

Antti Nuutinen, Yritysasiamies, University of Turku

KESKIVIIKKO / WEDNESDAY 29.03.2023
8:30 - 9:50

Session IX
Session host: Vilma Trapp

·30· 8:30

Verneri Nissilä

Promoter-specific effects of CarD, DksA on transcription in *Spirochaeta africana*

·31· 8:50

Josefiina Mäkinen

Äidinmaidon totaali IgA ja HPV16 spesifinen IgA – vaikutus lapsen HPV infektioon

·32· 9:10

Iida Payne

Selection and biophysical characterization of DARPins

·33· 9:30

Suvi Sulander

Ihmisen maitorauhasepiteelin haaroittumisrakenteen kuvantaminen ja kvantitointi

9:50 – 11:10 POSTER VIEWING *Pha 2*

11:10 – 12:30 LOUNASTAUKO / LUNCH BREAK

12:15 – 13:45 POSTER SESSION *Pha 2*

KESKIVIIKKO / WEDNESDAY 29.03.2023
14:05 – 15:25

Session X
Session host: Saara Östman

·34· 14:05

Elisa Lankinen

Development of a novel affinity maturation strategy utilizing error-prone PCR and mammalian display

·35· 14:25

Eetu Maaranen

Development of antibodies for non-competitive detection of saxitoxin

·36· 14:45

Akseli Jokela

Development of α-defensin lateral flow test for prosthetic joint infections

·37· 15:05

Niko Peltonen

Optimizing pre- and post-crosslinking methods for alginate using the Triaxial tool print head with microfluidic chips or mist sprayer

TORSTAI / THURSDAY 30.03.2023
8:30 - 9:50

Session XI
Session host: Selma Salonen

·38· 8:30

Veera Luukkonen

Detection and quantification of methionine 1-linked ubiquitin chains in intestinal inflammation and cancer models

·39· 8:50

Yasmin Alameldin

The role of Hippo regulators on Neuropathic pain

·40· 9:10

Niina-Elina Kärkkäinen

Development of blood and saliva sample preparation workflows for isothermal POC MDx assays

·41· 9:30

Juulia Allgaier

Establishment, characterization, and validation of dorsal root ganglia explant cultures for drug development

9:50 - 10:10 TAUKO / BREAK

TORSTAI / THURSDAY 30.03.2023
10:10 – 11:30

Session XII
Session host: Misba Chan

·42· 10:10

Oskari Nääjärvi

Development of lateral flow assay system with magnetic particles as solid support

·43· 10:30

Jasmin Toukoniemi

Selection platform for biparatopic DARPin-antibody fusion molecules

·44· 10:50

Shahrzad Bolouri

Novel antibody library design: Super stable disulfide bridge stabilized single chain variable fragment antibody library

·45· 11:10

Iines Auravuo

Affinity maturation of anti-gelsolin antibodies

11:30 – 12:30 LOUNASTAUKO / LUNCH BREAK

TORSTAI / THURSDAY 30.03.2023
12:30 – 13:50

Session XIII
Session host: Shamina Afrin

·46· 12:30

Narges Moradi

Generation of clinical diagnostic antibodies against common lymphatic endothelial and vascular endothelial receptor 1

·47· 12:50

Adeesha Herath

Isolation of rhinovirus-specific Fab antibodies from phage display antibody library

·48· 13:10

Sylvia Remes

Anti-EPLIN antibody development

·Alumni talk· 13:30

Ville Pollari, Analytical Scientist, DelSiTech

PERJANTAI / FRIDAY 31.03.2023
8:30 – 9:50

Session XIV
Session host: Sami Oksanen

·49· 8:30

Lucas Backström

Utilizing MALDI-TOF MS to distinguish clinically relevant strains of *C. diphtheriae*

·50· 8:50

Olli Kokko

CA125 glykovarianttimäärityksen muuntaminen magneettipartikkelipohjaiseksi kemiluminesenssimääritykseksi

·51· 9:10

Nea Laine

Geno1 teknologian automatisointi syöpädiagnostiikassa

·52· 9:30

Kaisa Leppä

Lateraalivirtausmääritys syöpämerkkiaineelle CA19-9 munasarjasyövän diagnostiikkaan

9:50 - 10:10 TAUKO / BREAK

PERJANTAI / FRIDAY 31.03.2023
10:10 – 11:30

Session XV
Session host: Kirsti Raiko

·53· 10:10

Maiju Liimatainen

Herkkä, leimakoetinkompleksin siirtoon perustuva määritys oligonukleotidianalyyttien havaitsemiseen

·54· 10:30

Rita Ojaniemi

Tunnistussilmukan kiinnittymisen ja sekvensointikirjastojen optimointi syövän diagnostiikassa

·55· 10:50

Eetu Välimäki

Funktionaalinen kasvainorganoidien viljelymenetelmä immuno-onkologisten lääkevasteiden tutkimuksessa

·56· 11:10

Helea Junes

DNA:n eristykseen ja puhdistukseen käytettävän kasetin valmistus 3D-tulostuksella infektiodiagnostiseen vieritestausjärjestelmään

11:30 – 12:30 LOUNASTAUKO / LUNCH BREAK

PERJANTAI / FRIDAY 31.03.2023
12:30 – 14:10

Session XVI
Session host: Saara Kuusinen

·57· 12:30

Tuulia Tuominen

Highly sensitive assay for long forms of cardiac troponin T to improve myocardial infarction diagnostics

·58· 12:50

Saara Sahlman

Nanoparticle aided glycovariant biomarkers for monitoring lung cancer

·59· 13:10

Emilia Kaipainen

Detecting cardiac troponin T forms in advanced chronic kidney disease patients

·Alumni talk· 13:30

Hanna Polari, Business Development Manager, PerkinElmer

·Closing Words· 13:50 **Prof. Eevi Rintamäki**

Ihmisen pluripotenttien kantasolujen indusoiminen ja karakterisointi

Sanne Forsback
Ohjaaja: Dos. Elisa Närvä
MOLEKYYLIBIOTIETEET, SOLUBIOLOGIA

Alkion kantasolut, joita kutsutaan pluripotenteiksi kantasoluiksi, pystyvät muodostamaan kaikkia kehon kudosten solutyyppejä. Näitä kehon erilaistuneita somaattisia soluja voidaan geneettisesti uudelleenohjelmoida alkion kaltaiseen tilaan, jolloin syntyy indusoituja pluripotentteja kantasoluja (engl. *induced pluripotent stem cell; iPSC*). Ihmisen somaattiset solut indusoidaan takaisin pluripotenttiin tilaan neljän uudelleenohjelmointitekijän avulla, Oct3/4, Sox2, Klf4 ja c-Myc; (OSKM). Uudelleenohjelmoinnissa OSKM yhdistelmän pakotettu ilmentyminen johtaa kromatiinin uudelleen järjestäytymiseen ja pluripotenttien kantasolujen muodostumiseen. Uudelleenohjelmointi tekniikan avulla voimme tuottaa potilasspesifisiä soluja ja täten lisätä ymmärrystämme eri sairauksien patogeneesistä ja kehittää uusia hoitomenetelmiä.
Tässä työssä genomiin intergroimattoman Sendai viruksen avulla tuotettujen iPS-solujen pluripotenssi, identiteetti ja turvallisuus selvitettiin uudelleenohjelmoinnin jälkeen erilaisten vakiintuneiden standardien mukaisesti. Pluripotenssimarkkeri analyysillä selvisi, että johdetut iPS-solut ilmensivät pluripotenttimarkkereita TRA-1-60, Nanog ja SSEA-4, sekä pystyivät muodostamaan alkiokantasolujen kertymiä, joista pystyttiin erottamaan ekto-, meso- ja endodermi suuntaan erilaistuneita soluja. Lisäksi todistettiin, että iPS-solulinjat ja alkuperäinen solulinja olivat geneettisesti identtisiä sekä karyotyypiltään normaaleja. Johdettujen iPS-solujen turvallisuus varmistettiin testaamalla Sendai viruksen ja mahdollisen mykoplasma kontaminaation negatiivisuus. Tuloksena saatiin iPS-soluja, joissa uudelleenohjelmointitekijät ovat sammuneet, ja joiden pluripotenssi, identiteetti ja turvallisuus on varmistettu.
Lisäksi uudelleenohjelmointi tekniikkaa testattiin munasarjasyövän potilasnäytteille. Munasarjasyövän solulinjat uudelleenohjelmoitiin geneettisesti modifioidun Sendai-virusvektoreiden ja OSKM tekijöiden avulla. Uudelleenohjelmoitujen solulinjojen morfologia muuttui kantasolumaiseksi, mutta solut eivät ilmentäneet pluripotenssimarkkereita, joten uudelleenohjelmoinnissa ei saavutettu täydellistä pluripotenttia tilaa. Tulevaisuudessa tavoitteena on käyttää iPS-soluja syövän ominaisuuksien ja kehityksen mallintamisessa.

Asiasanat: indusoidut pluripotentit kanta- ja syöpäsolut, uudelleenohjelmointi, karakterisointi

Molecular details of CIP2A-B56γ1 protein interaction

Nora Kullberg
Supervisors: Prof. Jukka Westermarck
MOLECULAR BIOSCIENCES, CELL BIOLOGY

Protein phosphatases and kinases are a crucial part of cellular signaling. Protein phosphatase 2A (PP2A) is a major serine/threonine phosphatase. The PP2A holoenzyme has three subunits: scaffolding A-, regulatory B- and catalytic C-subunits. The regulatory B-subunits determine the protein's function. Human B-subunits can be divided into four families: B55, B56, B72 and striatin. PP2A is a tumor suppressor, and it is inhibited in cancer mostly by non-genetic mechanisms. These mechanisms include post-translational modification and inhibition by PP2A inhibitor proteins such as cancerous inhibitor of PP2A (CIP2A).

CIP2A is an oncoprotein and its expression levels in normal tissues are low except in the testis. High expression levels of CIP2A have been reported in multiple cancer types. CIP2A binds to B56 subunits, and this way prevents A subunit from binding to B and C subunits. Further, by binding to B56 subunits CIP2A blocks the substrate binding pocket of B56. As CIP2A inhibits PP2A, the inhibition of CIP2A could be a therapeutical target.

N-terminal (1-560) fragment of CIP2A has been successfully purified and crystallized from E. coli, and it has been shown to interact with B56 proteins. Shorter N-terminal fragments of CIP2A have also been shown to interact with B56 proteins.

The aim of this project is to find the shortest N-terminal fragment of CIP2A that can bind and could be co-crystallized with B56γ1. All recombinant proteins were produced in E. coli. Binding assays of the proteins were conducted with microscale thermophoresis (MST) where fluorescent labelled proteins move directly through a microscopic temperature gradient. Changes in protein properties such as size and charge affect its movement through the temperature gradient.

We were able to prove with MST that CIP2A(1-330) binds to B56γ1. CIP2A(1-560) binding to B56γ1 was used as a positive control. The next step in the project is to test the binding of shorter fragments of CIP2A with B56γ1 and purify proteins for crystallization.

Keywords: protein phosphatases, protein phosphatase 2A, cancerous inhibitor of protein phosphatase 2A, microscale thermophoresis, protein crystallization

Onkogeenisen kRAS proteiinin toiminnan estäminen

Lotta Ristimäki
Ohjaajat: FT, Dos. Ulla Pentikäinen, FT Hanna Parkkola, FM Pekka Roivas
MOLEKYYLIBIOTIETEET, SOLUBIOLOGIA

KRAS (Kirsten rat sarcoma) on kRAS-proteiinia tuottava geeni. kRAS-proteiini kuuluu GTPaasi superperheeseen ja sen toiminta perustuu GTP:n sitoutumiseen. *KRAS*-geenin aktivaatio aikaansaa useita signalointireittejä, jotka säätelevät esimerkiksi solun proliferaatiota, erilaistumista ja selviämistä. Mutaatio *KRAS*-geenissä johtaa GTP:n sitoutumisen lisääntymiseen ja GTP:n hydrolyysin häiriöihin, jolloin kRAS-proteiini pysyy jatkuvasti aktiivisena. Tämä johtaa aktivaatioreittien toiminnan lisääntymiseen.
Mutaatiot *KRAS*-geenissä aiheuttavat noin 25 % kaikista syövistä ja se on helposti mutatoituva onkogeeni. Esimerkiksi 85–90 % kaikista haimasyövistä on onkogeenisen *KRAS*-geenin aiheuttamia. kRAS-proteiinin syöpää aiheuttavia mutaatioita ovat G12C, G12D, G12V ja Q61. Onkogeenisella kRAS-proteiinilla on uniikki reaktiotie, jonka takia syöpien hoito on vaikeaa. kRAS-proteiinin toimintaa säätelevien lääkkeiden kehitys on haastavaa, sillä proteiinista puuttuvat esimerkiksi tyypilliset lääkkeitä sitovat kohdat.
Työn tavoitteena on löytää molekyylejä, jotka estävät syöpää aiheuttavien kRAS-mutanttien toiminnan soluissa. Tässä tutkimuksessa keskitymme G12D ja G12V mutaatioihin. Tätä varten tuotamme villityypin kRAS ja kRAS-G12V-proteiinin GST-fuusiona *Escherichia coli*-bakteerissa. Työssä tuotamme ja puhdistamme itse kRAS-villityypin ja kRAS-G12V-proteiinin. Proteiinit puhdistetaan yhdistämällä affiniteetti- ja kokoerottelukromatografiaa. kRAS-G12D-proteiini on kaupallinen. Yhteistyökumppanin suunnittelemien molekyylien sitoutumista KRAS proteiineihin seurataan mikroskaala termoforeesin (MST) avulla.
Proteiinien tuotto- ja puhdistus ovat onnistuneet ja seuraavaksi on tarkoitus edetä molekyylien sitoutumisvoimakkuuden määrittämiseen.

Asiasanat: kRAS, onkogeeninen KRAS, GTPaasi, kRAS-WT, kRAS-G12D, kRAS-G12V

Monosyyttiaktivaatiotesti pyrogeenien testausmenetelmänä

Ellinoora Koivula
Ohjaajat: FT Paula Pennanen, FT Tuomas Nikula
MOLEKYYLIBIOTIETEET, SOLUBIOLOGIA

Pyrogeenit ovat kuumetta aiheuttavia aineita, jotka kehoon ja verenkiertoon päästessään voivat aiheuttaa vakaviakin oireita. Yleisimpiä pyrogeenejä ovat gramnegatiivisten bakteerien soluseinien lipopolysakkaridit. Lipopolysakkaridit ovat endotoksiineja, mutta niiden lisäksi on myös muita pyrogeenejä, kuten parasiitit tai virusten ja grampositiivisten bakteerien osat.

Lääketuotteet ja lääkinnälliset laitteet on testattava pyrogeenien varalta ennen niiden kaupallistamista. Perinteisimmässä pyrogeenien testaustavassa käytetään kaneja koe-eläiminä. Testin heikkouksia koe-eläinten käytön lisäksi on sen alhainen sensitiivisyys sekä ei-kvantitatiivisuus. Toinen usein käytetty testi on Limulus amebosyytti lysaatti -testi, joka on *in vitro* -testi, mutta sen heikkoutena on sen kyky havainnoida pelkästään endotoksiineja.

Monosyyttiaktivaatiotesti on edellä mainittuja pyrogeenien testimenetelmiä uudempi versio. Se sisällytettiin Euroopan farmakopeaan vuonna 2010 ja vuonna 2016 suositeltiin kanien käytön korvaamista monosyyttiaktivaatiotestillä aina sen ollessa mahdollista. Yhdysvaltain farmakopean mukaan validoitua monosyyttiaktivaatiotestiä voidaan käyttää perinteisten testimenetelmien tilalla, kun se on tarkoituksenmukaista.

Työssä käytettävä versio monosyyttiaktivaatiotestistä hyödyntää ihmisen Mono-Mac-6 monosyyttisolulinjaa sekä niiden tuottamaa sytokiiniä interleukiini 6:ta. Monosyyttisoluilla on solun pintareseptoreina Tollin kaltaisia reseptoreja (engl. *Toll-like receptor*, TLR). Reseptori tunnistaa pyrogeenin, jolloin sen konformaatio muuttuu ja tunnusomainen signalointireitti aktivoituu. Lopulta reitti johtaa ydintekijä-kappa B:n (engl. *nuclear factor kappa B*, NF-κB) ja MAP-kinaasin aktivaatioon. NF-κB siirtyy tumaan ja ilmentää spesifisiä geenejä, jotka indusoivat proinflammatorisia sytokiinejä, kuten interleukiini 6:ta. Monosyyttien mahdollisesti tuottama interleukiini 6 voidaan sitten mitata spesifisellä ELISA-immunomäärityksellä.

Asiasanat: pyrogeeni, monosyyttiaktivaatiotesti, interleukiini 6, TLR, ELISA

Immobilization of genetically engineered *Chlamydomonas reinhardtii* to improve photobiotransformation of cyclohexanone to ε-caprolactone

Tiia Siivola
Supervisors: Ph.D. Vilja Siitonen, Prof. Yagut Allahverdiyeva-Rinne
MOLECULAR SYSTEMS BIOLOGY

With an acute environmental crisis at hand, sustainable production processes are more crucial than ever. Photosynthesis offers an excellent solution because it takes atmospheric CO_2 up by utilizing solar energy to produce valuable compounds, which makes the process highly sustainable. A potential approach is to engineer photosynthetic micro-organisms and use them as cell factories. One cell factory application is to use the cells to perform photobiotransformation, where an external substrate is converted into a product using photosynthetically produced cofactors and oxygen. As a modern twist, the cell factories can be immobilized to solid-state thin-layer hydrogels, which enables a higher cell density, a more even light distribution to the cells, and a better volumetric production efficiency.
In this work, an engineered green alga *Chlamydomonas reinhardtii* possessing a cyclohexanone monooxygenase (CHMO) gene was used to produce ε-caprolactone from the fed substrate cyclohexanone *via* photobiotransformation. ε-caprolactone is a precursor of polycaprolactone, which is used, e.g., in biomedical applications. The strain was used to compare three cell immobilization approaches: alginate crosslinked with Ca^{2+}, TEMPO-oxidized cellulose nanofibrils (TCNF) crosslinked with Ca^{2+}, and UV-crosslinked, 3D-printed galactoglucomannan methacrylate (GGMMA) with alginate. Western blotting was used to find the most optimal timepoint for cell immobilization based on CHMO expression. ε-caprolactone formation was analyzed with gas chromatography and the production rate and duration between solid-state cells and a suspension were compared. Long-term production was tested in TCNF by performing photobiotransformations daily. The optimal timepoint for cell immobilization based on CHMO expression was after one day of growth in suspension, but since the reaction benefits from high chlorophyll content, the third day was the best compromise for immobilization. The photobiotransformation completed fastest in suspension, but the maximal rate, however, was highest in TCNF. Specific production rates based on chlorophyll and dry cell weight were also highest in TCNF. Long-term production was found to be successful, though the reaction slowed after a few photobiotransformation cycles. In conclusion, solid-state cell factories offer a unique and efficient platform for whole-cell photobiotransformation.

Keywords: cell factory, cell immobilization, CHMO, ε-caprolactone, microalgae, photobiotransformation, photosynthesis

Syanobakteerin Synechocystis sp. PCC 6803 sopeutuminen typen puutteeseen

Mithila Ray
Ohjaajat: Dos. Taina Tyystjärvi, FT Juha Kurkela
MOLEKYYLIBIOTIETEET, SOLUBIOLOGIA

Syanobakteerit ovat maailmanlaajuisesti laajalle levinneitä fotoautotrofisia prokaryootteja, jotka kykenevät muuttamaan auringon valoenergian avulla hiilidioksidin ja veden orgaanisiksi yhdisteiksi. Typpi on usein kasvua rajoittava ravinne fotosynteettisille organismeille. Malliorganismi *Synechocystis* sp. PCC 6803 (myöhemmin *Synechocystis*) on ei-diatsotrofinen laji, joka ei kykene sitomaan typpeä suoraan ilmakehästä, vaan ottaa typpeä nitraattina, ammoniumina tai ureana. *Synechocystis*-solut sopeutuvat typen määrän muutoksiin muuttamalla lukuisten geenien transkriptiota ja sopeuttamalla solun toimintaa. Vakavassa typen puutteessa kasvu loppuu ja valoenergiaa keräävät fykobilisomit hajoavat. Transkription säätelyyn osallistuvat RNA-polymeraasin sigmatekijät ja NtcA-transkriptiotekijä sekä yleinen typpimetabolian säätelijä, PII-proteiini.

Hiilen ja typen metabolia ovat liitoksissa toisiinsa ja niiden koordinoitu säätely on välttämätöntä solujen homeostasian ylläpitämiselle. SigC-sigmatekijästä, anti-SigC-tekijästä ja anti-SigC-tekijän vastavaikuttajasta (Ssr1600) koostuva signalointireitti yhdistää solujen kasvun käytettävissä olevaan hiilidioksidin määrään. Lisäksi hiilisignalointiin vaikuttaa RNA-polymeraasin ω-alayksikkö. Tämän tutkimuksen tavoitteena on selvittää vaikuttaako hiilisignalointireitti myös *Synechocystiksen* sopeutumiseen typen määrän muutoksiin. Tutkimuksessa käytettiin hiilisignalointireitin mutanttikantoja. ΔrpoZ kannalta puuttuu ω-alayksikkö ja se ei pysty sopeutumaan korkeaan hiilidioksidipitoisuuteen. Suppressorimutanttikannat ΔrpoZ-S1 ja ΔrpoZ-S2 pystyvät sopeumaan korkeaan hiilidioksidipitoisuuteen; molemmilla suppressorikannoilla Ssr1600-proteiinia on epätavallisen vähän. Kannassa ssr1600-oe genomiin on lisätty ylimääräinen *ssr1600*-geeni ja Ssr1600-proteiinia on tavallista enemmän. ΔsigC-kannalla ei ole toimivaa *sigC*-geeniä ja ΔsigBDE-kannalla taas ryhmän 2 sigmatekijöistä toimii ainoastaan SigC. Näiden kantojen kasvua ja pigmenttikoostumusta mitattiin solujen sopeutuessa typen puutteeseen ja toipuessa typen puutteesta. Tulokset osoittivat, että ω-alayksikkö, Ssr1600-proteiini ja SigC-sigmatekijä vaikuttavat *Synechocystiksen* sopeutumiseen typen puutteeseen. Lisäksi todettiin, että SigC-sigmatekijän runsaus hidastaa typen puutteesta toipumista.

AVAINSANAT: syanobakteerit, RNA-polymeraasi, sigmatekijä, typpi, hiili

Construction and evaluation of translationally optimized engineered production pathways in the cyanobacterium *Synechocystis sp. PCC6803*

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MOLECULAR SYSTEMS BIOLOGY

Recruiting microbial cells as cell factories for bio-production is not straightforward. Developing bio-based production systems involves engineering the target chassis, bioprocess optimization, and infrastructure development which takes much time, effort, and costs. In the stage of strain engineering, a multi-gene construct is assembled and delivered to be expressed in the target host in order to develop a microbial strain with the capability to produce the target product at a significant amount and efficiency. The DNA assembly is built by stitching multiple genetic parts (promotor, ribosome-binding site (RBS), etc) derived from different origins. Whether the protein itself was the target product or it catalyzes a step in an engineered pathway, heterologous gene expression is at the heart of biotechnological applications.
However, the performance of each genetic element cannot be predicted in a recombinant multi-gene construct which represents a lingering impediment to straightforward rational engineering of biological systems. This obstacle stems from the complexity of gene expression at transcriptional and translational levels and the myriad parameters that could affect it. In one approach, various expression constructs are assembled by combining variants of each genetic element followed by screening for optimal expression level. In another strategy, researchers attempt to remodel the DNA parts in a way that achieves consistent and predictable behavior in different contexts. Several designs have been assessed to get "functionally standardized" genetic parts, including isolated promotors, engineering a ribozyme or a CRISPR target sequence upstream to RBS, or refactoring biosynthetic gene clusters. These efforts were promoted by the recent advances in DNA assembly technologies such as Golden Gate, and Gibson assembly as well as the cost-effective de novo DNA synthesis which together facilitate the designing, constructing, and modifying of genetic parts. In our project "CynoConstruct", we have developed a golden-gate-based DNA assembly system where a multigene operon is efficiently assembled in a one-pot reaction. Unlike other published assembly platforms, RBSs are independent DNA parts in our system which allows gene expression optimization on the translational level by assessing RBS variants. Ethanol-producing constructs, differing in RBS combination, were assembled and transformed in Synechocystis sp. PCC 6803, then ethanol levels were measured by Gas Chromatography (GC-FID).

Keywords: Cyanobacteria, Ethanol production, Golden Gate assembly, Metabolic engineering, RBS-based optimization.

In vitro studies of the activity of a cholesterol-forming enzyme

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MOLECULAR BIOSCIENCES, CELL BIOLOGY

Cholesterol is a crucial sterol found in membranes of several animal tissues. It has many important endogenous roles, including acting as a structural component, taking part in membrane fusion, and as a precursor especially for bile acids, steroid hormones, and myelin. It is also involved in hedgehog signaling. Thus, cholesterol is vital throughout human life.

In the human body, cholesterol is synthesized in two pathways: Bloch and Kandutsch-Russell. Mutations in an enzyme taking part in these pathways is a major reason for a cholesterol deficiency disorder, which may be fatal for fetuses. In this disorder, cholesterol levels diminish, while toxic lipid levels rise. The enzyme responsible of this disorder can also be chemically inhibited. Specifically, drugs that inhibit it are considered to act as teratogens when women are exposed to them during pregnancy. This causes the same symptoms to develop in the fetus as the cholesterol deficiency disorder. However, there is a limited amount of experimental information about the drugs that inhibit this particular enzyme. Thus, screening for its inhibition during drug development could help detect drugs that may cause adverse and fatal outcomes during pregnancies.

This study aimed to (1) characterize and assess the suitability of human subcellular fractions for *in vitro* activity measurements of this enzyme, and also to (2) carry out a preliminary inhibition screening of pharmaceutical compounds which are known/suspected inhibitors of this enzyme. For this purpose, we used commercially available subcellular fractions together with thin-layer chromatography and liquid chromatography-tandem mass spectrometry methods.

Unexpectedly, our results showed that all three subcellular fraction matrices tested contain cholesterol, making it difficult to study the intended marker reaction. Hence, our attention was turned to an alternative reaction, catalyzed by the same enzyme. Interestingly, the product formed in this reaction was not found in neither of the tested subcellular fractions, suggesting that the second reaction may be more suitable for enzyme activity determination.

The study continues to examine the enzyme activity in the second pathway. If the pathway is suitable, we will carry out the preliminary inhibition screening using this pathway.

Keywords: cholesterol deficiency, cholesterol synthesis, drug development, enzyme inhibition, pregnancy, subcellular fractions, teratogenicity

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Molecular characterisation of PK-TF oncogenic gene fusions

Lopamudra Chatterjee
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MOLECULAR SYSTEMS BIOLOGY

Cancer is characterized by a variety of genetic alterations including missense, nonsense, frameshift mutations and chromosomal rearrangements. Chromosomal translocations often lead to oncogenic gene fusions, or oncofusions (OFs). Since their discovery, OFs have been extensively studied as potential tumour-specific drug targets in several cancer types. A recent systems level study has concluded that protein kinases (PKs) and transcription factors (TFs) are the two main gene families producing in-frame fusions resulting into fusion proteins. The aim of my project is to study the interaction and functional landscape of a few representative fusion proteins containing both protein kinase and transcription factor as fusion partners.

Protein-protein interactions (PPIs) play a pivotal role in disease networks and are highly dysregulated in diseased state. In the last few years, studying PPI networks has become a powerful tool for identifying the functional consequences of genetic variation. We used BioID and affinity purification coupled with liquid chromatography and mass spectrometry to capture and analyse both stable and transient interactors of fusion proteins. It had been previously hypothesised that fusion proteins deregulate cellular signalling by acquiring novel functions while losing most of their parent protein functions. Our results establish that fusion proteins indeed interact with a completely new cohort of interactors and most often end up in different sub cellular locations in comparison to their wild type counterparts. The results illustrate potential mechanisms via which fusion proteins can result in drastic changes in the biological functions of the affected cell population.

Keywords: affinity purification, BioID, fusion protein, mass spectrometry, oncofusion, protein-protein interaction

Hormonal regulation of the osteogenic signature in bone metastatic Castration-Resistant Prostate Cancer (CRPC)

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BIOSCIENCES - MOLECULAR SYSTEMS BIOLOGY

Bone is the most preferred site for prostate cancer (PCa) metastasis which is a significant cause of cancer-related death in men. Bone metastasis in PCa typically forms sclerotic lesions, i.e., an increase in abnormal bone mass, unlike, for example, breast cancer that primarily forms osteolytic lesions, reducing the bone mass. Currently, there are only palliative treatments available for patients with bone metastatic PCa. The aim of the present study was to investigate the effects of sex hormones on osteogenic bone formation in castration-resistant prostate cancer (CRPC) using a preclinical mouse model.

As a model, we used an intratibial VCaP xenograft in nude mice, mimicking the sclerotic phenotype of CRPC bone lesions. To study the contribution of adrenal-derived sex hormones, and their precursors, on the osteogenic signature, we compared sham and tumor-bearing bones in orchiectomized (ORX) and ORX+ adrenalectomized (ADX) mice. The transcriptome of the tumor-induced osteogenic signature (including, for example, *Bmp2, Bmpr1a, and Bmpr2*) was assessed by analyzing bulk RNA seq data annotated against the mouse reference sequence and confirming the RNA seq data with RT-qPCR. The data revealed that the osteogenic signature involved in tumor-induced bone formation observed in the ORX mice was attenuated in ADX mice. This suggests that the osteogenic progression in the CRPC model used is clearly dependent on adrenal-derived factors. We simultaneously examined the top annotated proteins secreted from the tumors, by annotating the RNA seq data against the human reference sequence to identify a potential tumor-derived inducer of the osteogenic response in the bone. The expression of the identified inducer in tumor cells was further planned to be analyzed by RT-qPCR and immunohistochemistry on the tumor bones.

Altogether, the data suggest that the adrenal-derived factors, likely steroid hormones, and their precursors, critically contribute to the osteogenic progression of CRPC *in vivo*.

Keywords: ADX, castration-resistant prostate cancer, ORX, osteogenic, prostate cancer.

Early detection of prostate cancer using Extracellular Vesicles (EVs) and NP-TRFIA

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MOLECULAR SYSTEMS BIOLOGY (TECH.)

Prostate cancer (PCa) is a type of cancer that affects the prostate, an organ in the male reproductive system. It is mostly diagnosed and deadliest cancers among men. In 2020, approximately 1.4 million new cases were diagnosed, causing 370,000 deaths. Although there are available techniques for detecting PCa, for example Prostate Specific Antigen (PSA), they have a set of drawbacks. In this study, we investigated whether utilizing a high-throughput method (FastEV^{TM}) for enriching extracellular vesicles (EVs) from clinical serum samples, in combination with nanoparticle-aided time-resolved fluorescence immunoassay (TRFIA) could enhance the accessibility of biomarkers for the early detection of PCa.

Extracellular vesicles (EV)- and protein enriched (PE)-fractions were separated from both PCa and benign prostate hyperplasia (BPH) patients using the FastEV technology. Biotinylated capture antibody was immobilized on streptavidin coated microtiter wells for capturing EV and PE-fractions of PCa. Then captured analyte was detected using glycan- binding lectin coated on NPs.

We have observed that assay consisting with cancer antigen (Ca15-3) and (Ca19-9) in combination with WGA lectin, such as Ca15-3^{WGA} and Ca19-9^{WGA} assays were able to significantly separate the PCa patients from the BPH sources (p-value = 0.007 and 0.00001), respectively.

In this study we have demonstrated that high-throughput method (FastEV^{TM}) along with simple nanoparticle-aided time-resolved fluorescence immunoassay (TRFIA) could be used to detect PCa patients from clinically challenged BPH conditions.

Keywords: prostate cancer, prostate specific antigen, extracellular vesicles, immunoassay, microtiter, capture, lectin

Hypoksian vaikutus matriksin metalloproteinaasien ilmentymiseen okasolusyöpäsoluissa

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SOLUBIOLOGIA

Matriksin metalloproteinaasit (MMP) ovat soluväliaineen rakenneproteiineja hajottavia proteolyyttisiä entsyymejä, jotka ovat tärkeässä roolissa kiinteiden kasvainten metastasoinnissa. Esimerkiksi epiteeli-mesenkyymi -muutos sekä ekstra- ja intravasaatio ovat riippuvaisia MMP:iden kyvystä hajottaa soluväliainetta. Okasolusyövässä on osoitettu ilmentyvän useita MMP:itä, jotka edistävät syöpäsolujen jakautumista ja syövän leviämistä.
Hypoksia eli alhainen happipitoisuus on tyypillinen piirre kiinteissä kasvaimissa, ja sen on todettu vaikuttavan muun muassa syövän invasiivisuuteen sekä metastaattisuuteen. Yksi tärkeä happimetabolian säätelijä on hypoksian indusoima tekijä 1α (HIF-1α), jonka on havaittu säätelevän esimerkiksi MMP:iden ilmentymistä useissa eri syövissä. Okasolusyövässä hypoksian vaikutusta ja sen vaikutusmekanismeja MMP:iden ilmentymiseen ei kuitenkaan ole vielä tutkittu, joten tämän tutkimuksen tavoitteena on selvittää miten MMP:iden ilmentyminen muuttuu hypoksiassa, sekä mitkä tekijät säätelevät sitä.
Tässä tutkimuksessa käytettiin yhdistelmäviljelmiä, joissa kahta eri okasolusyöpäsolulinjaa (RT3 ja UT-SCC-7) kasvatettiin yhdessä ihmisen ihon fibroblastien (engl. *normal human dermal fibroblasts*, NHDF) kanssa kolmiulotteisina kasvatuksina, sferoideina. Sferoidit mallintavat 2D-kasvatuksia realistisemmin kasvainten toimintaa ja syövän mikroympäristön vaikutusta soluihin. Useiden eri MMP:iden ilmentymistä tutkittiin sferoideista sekä proteiinitasolla että RNA-tasolla, ja tulokset viittaavat MMP:iden ilmentymisen olevan poikkeavaa hypoksiassa. Proteiinitasolla ilmentymistä tutkittiin kahden eri hypoksiamenetelmän avulla ja alustavat tulokset tukevat toisiaan. Esimerkiksi MMP-1:n ja MMP-2:n havaittiin lisääntyvän hypoksiassa. Myös RNA-tasolla alustavat tulokset viittaavat MMP-1:n määrän lisääntyvän hypoksiassa, kun taas MMP-10:n ja MMP-13:n ilmentyminen on vähentynyt. HIF-1α:n rooli tutkittavien MMP:iden ilmentymisen säätelyssä on vielä selvittämättä.
Tutkimuksesta saadut tulokset luovat perustan jatkotutkimuksille, joissa selvitetään eri MMP:iden ilmentymistä okasolusyöpäkasvaimissa. Tulokset voivat mahdollisesti tulevaisuudessa johtaa uusien biomerkkiaineiden käyttöönottoon okasolusyövän havaitsemisessa ja aggressiivisuuden arvioinnissa.

Asiasanat: hypoksia, MMP, okasolusyöpä, sferoidit

Comparison of prime editing and base editing for generating the novel WRN^{R732P} mutation in breast cancer cells

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MOLECULAR BIOSCIENCES, CELL BIOLOGY

Breast cancer is the most common cancer among women in Finland and worldwide, but, fortunately, the survival rate in Finland is over 90 %. Genome instability and mutations are one reason for the formation of cancer. WRN is one of the five RecQ helicases and has an important role in maintaining genome stability.
Prime editing and base editing are CRISPR-Cas9 genome editing methods that don't require double-strand breaks or donor DNA. Prime editing requires a prime editor (PE) composed of a catalytically impaired Cas9 endonuclease linked to reverse transcriptase (RT) and green fluorescent protein (GFP). The PE is directed to the desired editing site by engineered prime editing guide RNA (epegRNA), which also carries the template with the desired edit. Base editing requires a base editor (BE) and a guide RNA (gRNA) for guiding the BE to the correct site.
The aim of the thesis was to generate the novel WRN^{R732P} mutation into breast cancer cells using prime editing and base editing and compare which of the methods gives the highest editing efficiency. Three different primer binding site (PBS) and reverse transcription template (RTT) lengths were tested to determine the optimal PBS and RTT lengths for this specific locus. Two gRNAs were used to nick the non-edited strand in the prime editing constructs to favor the edited strand as a template in the repair process. CAL-51 cells were transfected with plasmids for the prime editor, epegRNA, gRNA, and hMLH1dn for prime editing, and CGBE1 base editor and CGBE gRNA for base editing, and GFP-positive cells were sorted. Editing efficiency was measured with droplet digital PCR (ddPCR) assay to determine the fractional abundance of the mutant cells versus the wild type. Both sgRNAs in prime editing worked but nicking further away from the edit site overall worked better. Editing efficiency was at its highest at 36,9 ± 6,2 % with PBS of 10 nucleotides (nt) and RTT of 22 nt. The trends for prime editing were clear: the longer the epegRNA, the worse the editing efficiency. Base editing had a 5,7 ± 4,6 % editing efficiency proposing that the first version of the C-to-G base editor still needs optimizing. In conclusion, base editing can be used to generate the WRN^{R732P} mutation but optimizing the PBS and RTT lengths for the specific locus will result in higher editing efficiency.

Keywords: breast cancer, WRN, prime editing, base editing

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Clever-1 interference to overcome resistance to immune checkpoint inhibitors

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MOLECULAR SYSTEMS BIOLOGY

The focus of this study was to determine if extracellular vesicles (EVs) derived from macrophages that express common lymphatic endothelial and vascular endothelial receptor-1 (Clever-1) give rise to immune checkpoint inhibitor (ICI) resistance and whether this resistance could be bypassed by Clever-1 interference. ICIs are therapeutic antibodies that target receptors on T cells and their ligands on antigen-presenting cells to stimulate the patient's own immune system. ICI targets include programmed death receptor 1 (PD-1) and programmed death ligand 1 (PD-L1) that normally restrain the activation of acquired immunity. Resistance to ICIs is known to arise from the immunosuppressive tumor microenvironment, which is maintained by a macrophage subset that expresses Clever-1.

EVs are shed by all cells and they originate from the endosomal pathway or budding of the plasma membrane. Due to this, they contain proteins along with other biomolecules that are expressed or endocytosed by the cell of origin. Thus, EVs derived from Clever-1+ macrophages may influence the efficacy of ICIs as Clever-1+ macrophages do.

The aims of this thesis were to study if Clever-1+ EVs suppress the effect of anti-PD-1 and anti-PD-L1 ICI antibodies and whether this suppression could be reversed by therapeutic Clever-1 blockade using the anti-Clever-1 antibody FP-1305 or specific Clever-1 knockdown and knockout using siRNA and CRISPR/Cas9, respectively. The ability of EVs to suppress ICI-mediated T-cell activation was studied using a cell-based PD-1/PD-L1 luciferase reporter assay. The EVs were isolated from either KG-1 cell or primary macrophage cultures by fractioning conditioned cell culture mediums with size exclusion chromatography to obtain samples containing only EVs.

Clever-1+ EVs suppressed ICI-mediated T-cell activation in the PD-1/PD-L1 assay. However, using the therapeutic antibody FP-1305 to block Clever-1 on EVs did not overcome the immunosuppressive effect of EVs. Using siRNA to knock down Clever-1 from KG-1-cell-derived EVs did not show improvement in overcoming the resistance to ICIs, but using CRISPR/Cas9 to knock out Clever-1 from primary-macrophage-derived EVs did. Taken together, it appears that Clever-1 blockade cannot overcome the immunosuppression caused by Clever-1+ vesicles, suggesting that FP-1305 cannot block Clever-1 on the EV surface.

Keywords: Clever-1, immune checkpoint inhibitors, extracellular vesicles

Comparison of information dependent acquisition and sequential window acquisition of all theoretical mass spectra to characterize dynamics of faecal novel conjugated bile acids in relation to islet cell autoantibodies

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Biosciences, MOLECULAR SYSTEMS BIOLOGY

Untargeted mass spectrometry is a powerful analytical technique for the identification and quantification of metabolites in complex biological materials. Two untargeted data acquisition namely information dependent acquisition (IDA) and data independent acquisition method such as: sequential window acquisition of all theoretical mass spectra (SWATH) can generate MS2 spectra in high resolution liquid chromatography mass spectrometry platform. However, each acquisition has advantages, limitations and their own strategy to select precursors for fragmentation. Depending on the analyte of interest, selection of suitable and sensitive acquisition method can increase the detection efficiency of analyte obtaining valuable information. Structural studies on bile acids have revealed that the microbial enzyme-bile salt hydrolase can conjugate bile acids with various amino acids. Such novel bile acid amidates (BAAs) are found to be associated with various health related phenotypes. The efficiency of detecting BAAs by IDA and SWATH and its link with type 1 diabetes (T1D) progression is still unknown.
In this study, firstly we developed and optimized IDA and SWATH method in SCIEX Triple TOF 6600 to compare detection efficiency of 110 BAAs. Secondly, with the optimized method we analyzed and quantified the presence of 110 BAAs in total of 305 fecal samples of children participating in DIABIMMUNE study. DIABIMMUNE study mainly focuses on finding the relation between hygiene or exposure pathogen and autoimmune disease; T1D and samples involved in our study were collected at longitudinal time point at 3, 6, 12, 18, 24 and 36 months of age from children who develop multiple islet cell auto-antibodies (ICAs), single ICAs and the control group with negative ICAs during follow-up.
Our result showed that IDA outperforms SWATH with detection of 98 and 57 MS2 spectra out of 110 BAAs respectively. Several BAAs primarily conjugated cholic acids were discovered in the faecal samples. However, quantitative analysis is still in progress and their relationship with ICAs and T1D progression is yet to be determined.

Keywords: bile acid amidates, bile salt hydrolases, data acquisition, gut microbes, liquid chromatography mass spectrometry, IDA, SWATH, T1D


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Sensory characteristics and phenolic profiles of the first generation interspecific hybrid strawberries

Carla Vecenâncio da Silva
Supervisors: D.Sc. (Tech) Niina Kelanne, Doc. Oskar Laaksonen, Assist. Prof. Maaria Kortesniemi
FOOD DEVELOPMENT

Strawberry (*Fragaria* × *ananassa*) is an important berry widely consumed in Finland and many other parts of the world. The challenges of its cultivation involve many factors, including weather conditions and diseases. Several studies have been conducted in order to develop new cultivars to overcome these problems. Breeding is also a means of achieving bigger berries that are more resistant to transportation and that have a more intense red colour, sometimes in detriment of odour and flavour attributes.

This study aims at investigating the differences between new strawberry hybrids and traditional strawberry cultivars by analysing selected sensory and chemical properties. Interspecific strawberry hybrids were a result of the cross between *F. chiloensis* and *F. virginiana*, developed by the NORDFRUIT breeding program. The descriptive sensory panel had a total of nine panellists evaluating six samples: five hybrids and one commercial cultivar. Fifteen odour, appearance and flavour attributes were analysed through three parallel evaluation sessions. The phenolic compounds were analysed with UHPLC-DAD, allowing the identification of different phenolic groups, such as flavonols and anthocyanins.

The statistical analysis will include the analysis of variance (ANOVA) and principal component analysis (PCA). Random Forest and other machine learning methods will also be used to find the most important variables for the generalisation model. The combination of all these methods will contribute to finding the most important correlations and differences of the sensory and chemical properties of strawberry hybrids and traditional cultivars.

Sensory evaluation results showed the hybrid strawberries had higher mean values for the total intensity of flavour when compared to the commercial strawberry cultivar. At the same time, these hybrids had lower intensities of the red colour. The results for the new hybrids also indicated a higher apparent viscosity and higher rate of seeds per surface area, the later contributed to higher graininess as a mouthfeel. Selection of new cultivars that satisfy both weather and disease resistance requirements, as well as keeping the most attractive sensory and chemical properties of strawberries is key to its commercial success.

Keywords: anthocyanins, descriptive sensory panel, flavonols, *Fragaria* × *ananassa*, HPLC-DAD-MS, phenolic compounds, plant breeding, strawberry

Pastan rakenteelliset ja aistinvaraiset ominaisuudet teollisessa kuumennusprosessissa

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ELINTARVIKEKEHITYS

Elintarvikkeena pastaa käytetään runsaasti ympäri maailman ja sitä valmistetaan yli 10 miljoonia tonneja vuosittain. Suomessa pastaa kulutetaan muuhun maailmaan verrattuna melko vähän, kuitenkin 3,2 kiloa asukasta kohden vuositasolla. Tällä hetkellä valtaosa Suomen markkinoilla olevista pastaa sisältävistä aterioista on kuivatuotteita tai eineksiä.
Pastataikina koostuu yleensä (durum)vehnäjauhoista ja vedestä; joskus joukkoon lisätään myös kananmunaa. Taikinan tärkkelys ja gluteeni ovat merkittäviä tekijöitä pastan kypsennyskäyttäytymisessä ja siksi vaikuttavat lopputuotteen ominaisuuksiin. Pastaa voidaan tehdä kokonaan tai osittain myös muista jauhoista, esimerkiksi kikherne-, linssi-, kaura- tai maissijauhoista. Koska tärkkelys ja proteiinikoostumukset eroavat vehnästä, näistä aineksista valmistetut pastat käyttäytyvät kypsennettäessä eri tavalla kuin vehnäpasta.
Työn tavoitteina oli löytää aistinvaraisten arviointien menetelmiä käyttämällä pastasäilykkeisiin soveltuvia, teollisen lämpökäsittelyn kestäviä pastoja, sekä tekijöitä, jotka edesauttavat niiden toimivuutta säilykkeen valmistusprosessissa. Tavoitteena oli myös selvittää mitkä asiat vaikuttavat pastan kypsennys-käyttäytymiseen.
Aistinvaraisissa arvioinneissa talon sisäinen raati arvioi teollisesti lämpö-käsiteltyjen pastojen ja pasta-annosten pastojen rakennetta, kypsyyttä, makua ja flavoria sekä muita ominaisuuksia miellyttävyys-, intensiteetti- ja ominaisuus-kartoitustesteillä.

Asiasanat: pasta, säilyke, tuotekehitys, aistinvaraiset arvioinnit

Kasvipohjaisten elintarvikkeiden aminohappokoostumuksen tutkiminen

Katariina Kumpula
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ELINTARVIKEKEMIA

Kasvipohjaisten elintarvikkeiden käyttö ruokavaliossa on jatkuvassa kasvussa. Kulutuksen kasvuun on sekä terveydellisiä, että ympäristöön liittyviä syitä, jotka myös tukevat lihan kulutuksen vähentämistä. Vielä ei kuitenkaan ole riittävästi tutkimustietoa siitä, kuinka hyvin kasvipohjaiset, erilaisilla elintarviketeknologian prosesseilla valmistetut tuotteet voivat ravitsemuksellisesti korvata lihatuotteita. Siksi niiden biokemiallisen koostumuksen tutkiminen on tarpeellista.
Tämä tutkimus on osa laajaa tutkimuskokonaisuutta, jonka tarkoituksena on selvittää kasviperäisten proteiininlähteiden terveysvaikutuksia keskittyen erityisesti niiden biokemialliseen koostumukseen ja uuselintarvikkeiden kehittämiseen (NewPlant). Tämän tutkimuksen tavoitteena oli analysoida kasvipohjaisia tuotteita kromatografisilla menetelmillä niiden aminohappokoostumuksen määrittämiseksi. Materiaaleina käytettiin kaupallisia kasviperäisiä elintarvikkeita, jotka oli tuotettu erilaisilla elintarviketeknologian prosessointimenetelmillä eri kasvipohjaisista raaka-aineista (esim. soija, herne). Näytteitä esikäsiteltiin emäs- ja happohydrolyyseilla, jotta aminohapot saataisiin vapautumaan proteiineista. Käsittelyä jatkettiin näytteiden laimentamisella ja derivatisoinnilla, joka tehtiin käyttäen o-ftalaldehydiä (OPA), fluorenyylimetyloksikarbonyylikloridia (FMOC-Cl) ja jodietikkahappoa (IDA). Tämän jälkeen näytteet analysoitiin fluoresenssidetektorilla varustetulla nestekromatografilla (UHPLC-FLD). Aminohappojen määritykseen käytettiin sisäisiä ja ulkoisia aminohappostandardeja.
Alustavien tulosten perusteella on havaittu eroja eri kasviproteiininlähteiden aminohappokoostumusten välillä. Myös prosessointitavan uskotaan vaikuttavan tuotteiden aminohappokoostumukseen. Tutkimus on yhä kesken, eikä kaikkia tähän mennessä saatuja tuloksia ole vielä käsitelty kokonaan.

Avainsanat: aminohapot, kasviperäinen, kasviproteiini, lihankorvike, nestekromatografia

Alkoholittomien oluiden aistinvaraiset laatutekijät

Tuomas Rysä
Ohjaajat: Dos. Oskar Laaksonen ja D.Sc Niina Kelanne
ELINTARVIKEKEMIA

Alkoholittomien oluiden (oluet, joiden alkoholipitoisuus alle 0.5 %) kulutus ja valmistus ovat jatkuvassa kasvussa ympäri maailman. Alkoholiton vaihtoehto valitaan useimmiten terveyssyistä ja alkoholin kulutuksen vähentämiseksi, sillä kuluttajat ovat yhä tietoisempia alkoholin kulutukseen liittyvistä riskeistä. Kuitenkin alkoholittomien oluiden valmistuksessa on yhä ongelmia saavuttaa aistein havaittavilta ominaisuuksiltaan sellaista olutta, joka vastaisi täysin perinteistä alkoholipitoista olutta. Alkoholittomissa oluissa on aiemmissa tutkimuksissa havaittu olevan epämiellyttäviä sivumakuja, sekä oluita on kommentoitu olemattomasta rungosta ja latteasta mausta.

Tutkimuksen tavoitteena oli selvittää kaupallisten alkoholittomien oluiden aistittavan laadun ominaisuuksia käyttäen kaasukromatografi-olfaktometria (GC-O) koulutetulla raadilla oluiden haihtuvien hajuyhdisteiden analysointiin ja tunnistamiseen. Tällä menetelmällä saatiin kerättyä samanaikaisesti tietoa haihtuvien yhdisteiden hajujen ominaisuuksista ihmisarvioijalla, sekä kromatografisesti. Oluiden haju- ja makuominaisuuksia tarkasteltiin lisäksi kuluttajaraadin (n=76). Kuluttajaraadissa menetelminä käytettiin pääasiassa choose-all-that-apply (CATA) ja rate-all-that-apply (RATA) menetelmiä tiedonkeruuseen. Maku- ja hajuominaisuuksiksi valittiin oluille tyypillisiä kuvailuja, kuten maltaisuus, happamuus ja hedelmäisyys. Olutnäytteinä käytettiin eri maalaisia ja eri panimoiden valmistamia lager-, pils-, ja Indian Pale Ale (IPA) -oluita.

Tutkimuksessa alustavien tulosten perusteella oluiden aistinvaraisissa ominaisuuksissa on tilastollisesti merkittäviä eroja tietyissä ominaisuuksissa, esimerkiksi kuluttajaraadin aistinvaraisessa arvioinnissa yhdessä IPA-oluessa oli muita oluita selvästi voimakkaampi karvas maku. Vaikka osassa näytteitä oli tuoteselosteen mukaan alkoholia 0,3 %, oli luultavasti määrä sen verran vähäinen, ettei kuluttajaraati löytänyt lainkaan tilastollisesti merkittäviä eroja 0,0 % oluiden kanssa. Tulosten käsittely on kuitenkin vielä kesken.

Tutkimustieto alkoholittomien oluiden saralta on tarpeen, jotta tuotteet kykenisivät vastaamaan paremmin kuluttajien mieltymyksiin, esimerkiksi kehittämällä makua tai hajua vastaamaan enemmän perinteistä alkoholipitoista olutta.

Asiasanat: aistinvarainen arviointi, alkoholittomat oluet, GC-O, haihtuvat yhdisteet

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Fatty acid composition of lipids in marine products, milk, and oils

Nita Pokharel
Supervisors: Ph.D. Marika Kalpio, Ph.D. Jukka-Pekka Suomela
FOOD DEVELOPMENT

Lipids are insoluble in water and soluble in organic solvents. Three main types of lipids comprises triacylglycerols (TAG), phospholipids and sterols. TAG is the major dietary lipid class, and is composed of three fatty acids (FAs). Different types of FAs are available from natural sources ranging from 4 to 30 carbons and 0 to 6 double bonds. FAs serve as a major energy source and a membrane-building molecules in a living cell. Different FAs have different roles and health effects. Therefore, it is important to study FA composition to assess the role of every FA.

This thesis intends to develop a method to separate, detect and quantify different FA methyl esters originally derived from different lipid sources. Lipid samples were selected so that they contain wide range of different FAs. For the quantitative analysis, a gas chromatography with flame ionization detector (GC-FID) (SHIMADZU GC-2030) was used and conditions were optimized. Similarly, the qualitative analysis method was developed by optimizing the gas chromatography with mass spectrometry detector (GC-MS) (Thermo TSQ8000Evo). For both chromatographic analyses DB-23 column (60 m, ID 0.25 mm, film thickness 0.25 μm) was used. Initially, the testing was carried out with GC-FID without the addition of internal standard.

The preliminary results showed that a dinonadecanoyl-phosphatidylcholine (19:0) can be used as an internal standard. Good separation with GC-FID was achieved after optimizing the oven temperature ramps for different sample types. The total run time accounted for 40.71 minutes. Similarly, with GC-MS instrument the best separation and fragmentation were obtained with optimized temperature and MS parameters. However, the major issue with the current GC-MS conditions was the fragmentation products with lower intensities, and further optimization is still continuing.

Keywords: lipids, fatty acids, GC-MS, GC-FID, method development, qualitative analysis, quantitative analysis

Health Effects and bioavailability of *n*−3 fatty acids

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FOOD DEVELOPMENT

Long-chain omega-3 polyunsaturated fatty acids (PUFAs) play crucial roles in various physiological processes in the human body. Among these, docosahexaenoic acid [DHA, 22:6(*n*−3)] is particularly essential for proper development and function of the brain and retina throughout life. Despite its importance, DHA deficiency has been widely reported worldwide due to the low intake of DHA and its low absorption. Recent research has shown that the distribution of fatty acids (FAs) within triacylglycerols (TAGs) affects lipid absorption.

With recent advances in the synthesis and determination of enantiospecific structured TAGs, this study was able to determine if dietary TAGs possessing DHA either in *sn*-1, *sn*-2, or *sn*-3 position and two palmitic acid residues in the remaining *sn*-positions, [*sn*-22:6(*n*−3)-16:0-16:0, *sn*-16:0- 22:6(*n*−3)-16:0 or *sn*-16:0-16:0-22:6(*n*−3)] would lead to the difference in FAs content or composition of visceral fat in rats. In addition to these three intervention groups, there were three control groups - tripalmitin, *n*−3 deficiency and normal feed. The study was conducted in male rats and the intervention time was 4 weeks. The experimental part focused on the extraction of lipids from visceral fat samples, followed by the analysis of FA methyl esters using gas chromatography. Variations in the FA content of the lipids in visceral fat were observed between all groups.

DHA showed a higher absorption on the *sn*-3 position of TAGs in visceral fat when compared with the DHA located on the *sn*-1 and 2 positions. Moreover, DHA located on the *sn*-3 position of structured TAGs had a higher content of FAs 20:1(*n*−9) and 20:4(*n*−6), and total (*n*−3) PUFAs in visceral fat TAGs compared with the *sn*-1 position. However, when DHA was situated at the *sn*-2 position as opposed to the *sn*-1 and *sn*-3 positions, there were no discernible disparities in the FA composition of visceral fat were discovered. This study showed the different bioavailability of DHA in different positions of TAGs.

Keywords: bioavailability, docosahexaenoic acid, stereospecific, triacylglycerol, visceral fat

Encapsulated linseed oil ethyl esters and microalgae oil for food fortification: Fatty acid and volatile profiles

Saida Samadova
Supervisors: PhD Annelie Damerau, Prof. Kaisa Linderborg
FOOD DEVELOPMENT

Linseed oil and microalgae oil are rich in omega-3 polyunsaturated fatty acids (*n*-3 PUFAs), alpha-linoleic acid (C18:3, ALA), and eicosapentaenoic acid (C20:5, EPA) and docosahexaenoic acid (C22:6, DHA), respectively. They are known for neuroprotective, anti-inflammatory and cardiovascular properties and play a crucial role in normal health. Nevertheless, *n*-3 PUFAs are easily oxidized based on their high level of unsaturation. Oxidation causes detrimental changes in taste, flavor, color, texture, and safety, reducing the nutritional value and sensory quality of the product. Encapsulation is an effective method for preventing oxidation of n-3 PUFAs and concealing off-flavors. Spray drying is the oldest and most common encapsulation technique due to its flexibility, durability, and cost-effective factors. Prilling is a recent technology that is mostly employed in the pharmaceutical industry utilizing ionotropic gelification.

The aim of this study is to investigate the influence of encapsulation of linseed oil ethyl esters on their fatty acid composition and release of volatiles. Encapsulations have been performed by spray-drying with various starches. Further, the influence of *in vitro* digestion of prilling-encapsulated microalgae oil on fatty acids will be investigated. Thus, the impacts of both processing and digestion will be studied.

The fatty acid profile of the linseed oil consisted of ALA (54.7%), oleic acid (19.2%), linoleic acid (16.2%), palmitic acid (5.5%) and stearic acid (2.7%), with other fatty acids being less than 1%. Production of ethyl esters and spray-drying had only minor effects on the fatty acid profiles. Volatile profiles were studied to investigate whether encapsulation could mask potential flavors. Both the starch type and the level of gelatinization affected the volatile profiles. Ethanol residues form ethyl esterification were detected. Native potato starch had the highest ethanol retention, followed by pregelatinized potato starch and pregelatinized rice starch. Encapsulation with maltodextrin resulted in the highest release of lipid oxidation compounds. The most promising formulation for food fortification applications in case of linseed oil ethyl esters was encapsulation with pregelatinized potato starch regarding fatty acid composition and maltodextrin based on ethanol retention and total volatile release. The data analysis of microalgae oil samples is ongoing.

Keywords: omega-3, oxidation, polyunsaturated fatty acids, prilling, spray-drying

LÖYDÄT SHOTIT MEHUHYLLYSTÄ

Marli

VAIKUTTAVAN HYVÄ SHOT

HELPPO ANNOS PÄIVITTÄISTÄ HYVINVOINTIA

VATSAN HYVIN-VOINTIIN*

Runsaasti hedelmä- ja kasviperäisiä **kuituja.**

MUISTIN TUEKSI*

B6- ja B12-vitamiinit edistävät normaaleja psykologisia toimintoja.

SYDÄMELLE*

Tyydyttymättömät **omega-3-rasvahapot** edistävät veren kolesterolitason pysymistä normaalina.

VASTUSTUS-KYVYLLE*

C-vitamiini vahvistaa vastustuskykyä ja auttaa vähentämään väsymystä.

*Huolehdi tasapainoisesta ja monipuolisesta ruokavaliosta sekä terveistä elämäntavoista.

Development of lingonberry wines with a sensomics approach

Shania Saini
Supervisors: Oskar Laaksonen (Ph.D. Doc.), Niina Kelanne (D.Sc. Tech)
FOOD DEVELOPMENT (TECH.)

Albeit the categorization of lingonberry as a superfruit courtesy of its bioactive composition, its valorisation has remained sub-par ascribed to a challenging flavour profile. Masking these unpalatable attributes with excessive amounts of sugar has been the norm in the food industry; a counterproductive bane to human health. Additionally, fermentation to obtain lingonberry wines is non-existent due to the presence of a natural antimicrobial agent, benzoic acid.
The aim of this study was to employ baker's yeast mediated benzoic acid decrease, followed by conventional (*Saccharomyces cerevisiae*) and non-conventional (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) yeast strain fermentation to concoct medium alcohol content, non-clarified lingonberry wines; sans added sugars to preserve bioactive benefits. Next, sensomics profiling of the developed wines was conducted through volatile compounds' semi-quantification and sensory evaluation.
After benzoic acid decrement from 0.71g/L to 0.1g/L, wines with an average alcohol content of 7.7% (in incubator; IB) and 7.3% (at room temperature; RT) were created. Alteration of the perceived sensory intensities was detected between the lingonberry juice and wines as follows: a decrease in lingonberry flavour; and an increase in astringency, bitterness, ester odour, and alcohol odour. This was supported by an elevation in ester and higher alcohol contents, along with a decrease in terpene composition (except linalool and alpha-terpineol) in the wines. However, minimal statistically significant difference was observed in the sensomics profiles between varied yeast strain wines.
This illustrates that an effective benzoic acid reduction (below critical limit of 0.25g/L) facilitates fermentation that leads to a decrement of undesirable flavours that could potentially be capitalized upon; despite prolonging the processing time. Further, subsequent studies should optimize RT fermentations to minimize time and eliminate oxidation.

Keywords: benzoic acid, fermentation, lingonberry wine, sensomics, sensory evaluation, volatiles

Effect of yeast strains on the chemical composition of apple ciders from Finnish cultivars

Okwum ada obianuju
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FOOD DEVELOPMENT

Apple ciders are fermented acholic beverages (AFB) from apple juice with an alcohol concentration ranging from 1.2% to 8.5% they are created by partially or fully fermenting fresh or reconstituted fruit juice with or without the addition of sugar, water, or flavoring. However, it is a crucial consideration when choosing apple cultivars or how to combine different apple juice varieties to create a particular cider assortment as there are few studies on the properties of specific cultivars used in making ciders. This study is aimed at investigating the use and characteristics of native apple cultivars, and the effect of yeast strains on the residual sugars, ethanol, organic acids, and color properties of apple cider beverages.

Indigenous Finnish apple cultivars grown at St. Mary Parish Garden in Turku were provided by Natural Resources Institute Finland for this study. 2kg of each apple cultivar were harvested during autumn and stored at a cold temperature for ripening before processing. Juice extracted from the apples was inoculated separately with strains of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* yeast without added sugar and left to ferment for 30 days at 22°C. Ethanols, sugars, and organic acids were analyzed using gas chromatogram coupled with flame ionizaition detector (GC-FID).

S. cerevisiae yeast-fermented beverages had higher ethanol contents (2.57–7.67%, v/v) than those produced by *T. delbrueckii* fermentation (0.63- 6.26%, v/v). In general, the fermentation of apple juice increased the contents of organic acids succinic acid, malic acid, citric acid, and quinic acid whereas sugar contents decreased in all cultivars. All of the fermentations increased the formation of 3-methyl-1-butanol which impacts the final flavor profile of alcoholic beverages, even at low concentrations.

The result obtained from this experiment will provide new knowledge about the possibility of producing high-quality AFB from specific cultivars as well as offer technical advice to the beverage sector. Additionally, this study will encourage the commercial production of AFB using native Finnish apple cultivars.

Keywords: fermentation, inoculation, cider, AFB, yeast, *Saccharomyces cerevisiae, Torulaspora delbrueckii.*

Impact of *Saccharomyces* and non-*Saccharomyces* yeast on fermented vinegar from Finnish cultivar apple peels

Elsa Xifre Pujol
Supervisors: D.Sc. (tech.) Niina Kelanne, Doc. Oskar Laaksonen
FOOD DEVELOPMENT

In a world where population is continuously growing, natural sources are scare, and tons of food are being wasted, food security is guaranteed for only part of this population. Approximately 30% of food production is lost from harvest to retail and consumer consumption. Particularly, 21.6 % of worldwide fruit production is wasted. This is why, valorization of co- and by-products is arousing interest among food scientist. In this sense, fruit peels, seeds or pomace can be used as raw materials for new food products.

In this study, apple peels from Finnish cultivar were used to produce vinegar. In general, vinegar production involves two consecutive fermentations of the raw material. Firstly, an alcoholic fermentation, performed by yeast, and finally an acetic fermentation, performed by acetic acid bacteria.

The major aim of this study was to determine differences among apple peels vinegar fermented with *Saccharomyces* yeast (*Saccharomyces cerevisiae*), non-*Saccharomyces* yeast (*Torulaspora Delbrueckii*) and a sequential fermentation with non-*Saccharomyces* yeast (*Lachancea thermotolerans*) and *S. cerevisiae*.

Monitoring of alcoholic fermentation was done by weighting the samples. Total soluble solids content was analyzed using a refractometer, and ethanol content using GC-FID. For the acetic fermentation the monitoring was done by determining the pH of the samples, and ethanol and acetic acid content were analyzed also with GC-FID.

Results showed a difference among samples regarding final ethanol content after alcoholic fermentation, which was higher for samples inoculated with *S. cerevisiae* (7.49%), followed by *L. thermotolerans*/*S. cerevisiae* (7.07%) and finally *T. delbrueckii* (6.91%). Latest results from the acetic fermentation samples also show differences among yeast species regarding the final acetic acid content.

Keywords: apple peels, food waste, *Lachancea thermotolerans*, *Saccharomyces cerevisiae*, *Torulaspora Delbrueckii*, vinegar.

Functional screening of beneficial strains and consortia to maximize healthy fermentation potential

Zeynep Öztürk
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FOOD DEVELOPMENT

Food is pivotal to human survival. In response to the increasing population of the planet, humanity faces the challenge of adequate food production. It is necessary to follow more sustainable dietary patterns to supply enough protein and other nutrients to the growing population. People are more aware and looking for alternatives to the traditional animal-based diets that have dominated for centuries. Increasing concerns about the environmental impacts and food safety issues of industrial livestock farming is extending sustainability trends towards plant-based nutrition.

Legumes and cereals are two of the most important plant-based sources and are widely consumed globally. They are regarded as health-promoting due to their low-fat content, high protein content, dense dietary fiber content, and phosphate content. However, despite their high nutritional content, both legumes and cereals contain antinutritional factors and fermentable oligo-, di-, monosaccharides and polyols (FODMAP) that can interfere with the digestion and absorption of essential nutrients, leading to a reduced availability of these nutrients to the body. This thesis was a part of the EU Horizon project "Healthferm" (https://www.healthferm.eu/). This thesis was studied to screen available food fermentation lactic acid bacteria for optimum fermentation and health promoting effects. The aim is to evaluate the fermentation performance, growth kinetics, acidification, metabolite production, fiber degradation, anti-nutritional factors and FODMAPs in consortium and individual strains using a high throughput microreactor. In this study, oat flour, oat concentrate and faba bean concentrate based media were prepared and the growth kinetics of selected strains were examined and screened using Bioscreen. Growth curves of selected strains on cereal and legume-based media were plotted. It is anticipated that through the process of fermentation, the antinutrient factors and FODMAPs present in faba and oat will decrease, leading to improved digestion of minerals, vitamins and proteins.

Keywords: Plant-based, sustainable, fermentation, lactic acid bacteria, legume, cereal, antinutritional factors

Analysis of novel plant-based foods with ^{1}H Nuclear Magnetic Resonance spectroscopy

Wadiyo Samantha Hedwig

Supervisors: D.Sc. Kang Chen, Assist. Prof. Maaria Kortesniemi, Prof. Kati Hanhineva

FOOD DEVELOPMENT (TECH)

Plant-based diets are growing increasingly popular due to their potential health benefits, consumer concerns about the negative health effects of eating diets high in animal protein, and increased consumer awareness of the need to improve the environmental sustainability of food production. Meat analogues and substitutes have been developed to meet consumer demands and ensure the sustainability of future food supply, and the market has grown exponentially in recent years. The food industry is therefore continually evolving to meet the needs. This has heightened interest in research into the structure and formulation of plant-based meat alternatives, with the goal of determining their effectiveness as a substitute for protein and other nutrients abundant in animal meat. This Master's thesis study is a part of the Newplant study in the Erkko project aimed at analyzing novel plant-based foods using ^{1}H nuclear magnetic resonance (NMR) metabolomics. The study aimed at identifying the lipid composition of these foods and understanding how they differ from traditional animal-based foods.

In this study, polar lipids were extracted from 178 samples with methanol and chloroform. Each sample was analyzed utilizing ^{1}H NMR spectroscopy, and untargeted/targeted metabolomics approach was applied, and the NMR spectra analyzed using TopSpin, Chenomx and SIMCA software. The results focused on the relative comparison of integrals, NOVA classification, the product type, and protein source of the samples. Statistically significant results were obtained from all the samples with fava beans, peas, oats, and soy protein, particularly in highly and moderately processed products. Ball-type products and tofu showed significant bisallylic integrals (-CH=CHCH$_2$CH=CH-) indicating an abundance of polyunsaturated fatty acids with protons attached to bisallylic carbon. Ball and nugget samples, as well as products sourced from peas and oats showed abundant CH$_2$ glycerol backbone integrals (*sn1,3* triacylglycerols). This study will contribute to the growing field of plant-based food research and provide insight into the development of new, healthier food options.

Keywords: plant-based, novel foods, lipids, ^{1}H NMR spectroscopy, metabolomics

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Sensory characterization of commercial plant-based meat analogues

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Supervisors: Ph.D., Docent Oskar Laaksonen, Prof. Kati Hanhineva
FOOD DEVELOPMENT (TECH)

As consumers are increasingly interested in plant-based alternatives as dietary protein sources, the demand for products that closely mimic animal meat, especially in taste and texture has grown. While there are a few sensory studies on plant-based meat analogues which are focused on new formulations using functional ingredients or under-utilized plant proteins, there is little or no sensory information on different categories of commercial plant-based meat analogues. The objective of this study was to evaluate the sensory attributes of a variety of commercially available plant-based protein alternatives with focus on taste and texture.

The metabolomics results of the initial 168 commercial plant-based protein alternatives informed the selection of 16 samples from three major categories which are, products with whole legumes, products with whole legumes processed into food and products with legume protein concentrates/isolates. Nine samples from the processed categories were eventually analysed.

A total of 10 sensory panellists (6 females and 4 males) were trained to use descriptive analytical method to identify differences among commercial plant-based meat analogues. The panellists compared different taste and texture attributes of the samples to references on a line scale from 0 to 10 using the Compusense 20 software. Panel performance was investigated to obtain an established result. Multivariate analysis on the data showed that there was significant difference among all samples for both taste and texture attributes. Umami and saltiness had the most significant difference while sourness and astringency revealed the least significance for taste attributes. Similarly, rubbery showed the most significant difference while softness was the least significant for texture attributes. These differences in the attributes may be due to processing techniques employed, ingredients used in production of the meat analogues, or the type of plant protein utilized during processing. The study also revealed that only one out of the 9 samples was meat-like in taste and texture.

Therefore, results of this study will provide insights into the sensory characterization of these commercial products, guide plant-based meat analogue producers on attributes where improvement is needed and inform future development of novel plant-based meat analogues that better replicate the experience of eating animal-based meat.

Keywords: Sensory characterization, plant-based meat analogues, taste, texture, descriptive analysis, multivariate analysis.

Fermentation of lupin with lactic acid bacteria using a bioreactor

Malviina Nikola
Supervisors: MSc Jasmin Raita, D.Sc in Tech. Niina Kelanne, PhD Oskar Laaksonen, Prof. Kati Hanhineva
FOOD DEVELOPMENT

Lupinus angustifolius (*Lupinus* genus, Fabaceae family), also known as "blue lupin" or "narrow-leafed lupin", belongs to the group of sweet lupins due to its low level of bitter-tasting alkaloids and since it is safe for animals and humans to consume without risk of toxicity. The interest in using lupin as food has increased due to its unique nutritional value and potential health benefits, such as the relatively high protein content. Usually, lupine seeds are grounded into flour or utilized as such for a variety of food items.

Fermentation is a natural bioprocess commonly used to preserve food. In addition to increasing shelf life and microbiological safety, fermentation can be used to reduce toxicity and improve the nutritional values and sensory properties of raw materials and developed food products. Lactic acid bacteria produce lactic acid as the major metabolic product during fermentation. Although lactic acid bacteria fermentation is typically utilised for dairy products, it has shown promise for usage in the plant-based goods. The aim of this project is to examine the effect of lactic acid bacteria fermentation on dairy analogue made from lupin flour.

Lupin flour was mixed with tap water using a blender and liquid fraction was separated from solids by centrifugation. Three *Lactiplantibacillus plantarum* strains were used for fermentations. Test fermentations were carried out in centrifuge tubes for 24 h and 48 h at 30 °C in 15 ml batches. First fermentation using a bioreactor was done for 530 ml batch for 48 h at 28 °C. Samples were taken out at the different timepoints and pH was measured from samples and simple sugars and organic acids were analysed quantitively using gas chromatography with flame ionization detector.

Preliminary results show that fermentation significantly affects the contents of sugars and acids. Sugar content decreased during fermentation and lactic acid was formed in the liquid lupin fraction. Next steps of the project include more fermentations with bioreactor.

Keywords: bioreactor, fermentation, GC-FID, lactic acid bacteria, lupin

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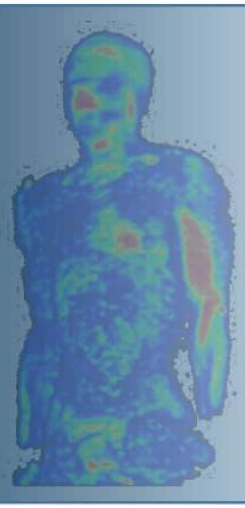

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Promoter-specific effects of CarD, DksA on transcription in *Spirochaeta africana*

Verneri Nissilä
Supervisor: Docent Georgi Belogurov
BIOCHEMISTRY

Selective transcription (DNA-templated biosynthesis of RNA) catalysed by RNA polymerase (RNAP) is the basis of differential gene expression. Generally speaking, transcription can be divided to three phases: initiation, elongation, and termination, all of which can be activated or repressed by specialised proteins known as transcription factors (TFs). Here, the effects of two TFs (CarD and DksA) on *Spirochaeta africana* promoters were studied. *S. africana* is a model organism for pathogenic spirochaetes, such as *Treponema pallidum*, the causative agent of syphilis. Diseases caused by spirochaetes are often chronic and remain poorly understood, increasing interest in spirochaetal molecular biology.

Here, transcription templates encoding one of the studied promoters (the Gre promoter P_{Gre}, and the ribosomal promoter P_{rrna}) and a fluorogenic RNA aptamer were used to monitor transcription. Transcription reactions were assembled *in vitro* and their resultant fluorescences were observed for 10 minutes. To see the effect of CarD and DksA on the promoters, reactions with an excess amount of transcription factors were assembled and their fluorescences compared to reactions where no additional factors were used.

CarD increased the end point fluorescence from P_{rrna} by around twofold as compared to the holoenzyme alone; reactions with DksA resulted in fluorescences 20-25% lower than baseline. Baseline transcription from P_{Gre} was threefold higher than that from P_{rrna}. Interestingly, DksA did not influence transcription from P_{Gre}. Finally, CarD inhibited transcription from P_{Gre}, with the observed end point fluorescences between 40-50% lower than with holoenzyme alone.

These results indicate that CarD upregulates transcription of P_{rrna} when no other regulatory elements are considered, whereas DksA suppresses it; P_{Gre}, on the other hand, is suppressed by CarD, while DksA doesn't seem to have much of an effect. Different combinations of these two factors, then, could function as a metabolic switch, together determining the relative activities of the promoters. Biochemically, such a mechanism might be important in the orchestration of global changes in metabolism, such as in the transitioning to the stringent response under unfavourable conditions, or activation of pathogenesis.

Keywords: promoter, DksA, CarD, *Spirochaeta africana*, transcription factor

Äidinmaidon totaali IgA ja HPV16 spesifinen IgA – vaikutus lapsen HPV infektioon

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Ihmisen papilloomavirukset (human papilloma virus, HPV) ovat yleisiä limakalvo- ja ihoinfektioita aiheuttavia DNA-viruksia. HPV infektio on usein oireeton ja paranee spontaanisti, mutta joissain tapauksissa infektio voi kroonistua. HPV-virukset voidaan jakaa suuren ja pienen riskin HPV-tyyppeihin niihin liittyvän syöpäriskin mukaan. Kroonistuneella suuren riskin HPV-infektiolla, esimerkiksi HPV16, on vahva yhteys erilaisten syöpien, kuten kohdunkaulan syövän syntyyn. HPV16 tartunta saadaan usein sukupuoliteitse, mutta lapsi voi saada tartunnan äidiltään esimerkiksi synnytyksen yhteydessä. Niin aikuisilla kuin lapsilla virus voi infektoida sekä genitaalialueen- että suun limakalvoja.
Lapsen immuunijärjestelmä kehittyy täysin toimivaksi vasta syntymän jälkeen ja äidinmaidon sisältämät vasta-aineet antavat lapselle tarvittavan suojan infektioita vastaan, minkä lisäksi ne vaikuttavat merkittävästi lapsen immuunijärjestelmän kehitykseen ja säätelyyn. Tämän tutkimuksen tarkoituksena on selvittää, erittyykö HPV16 vasta-aineita äidinmaitoon ja onko niillä vaikutusta lapsen HPV-infektion syntyyn ja kehitykseen, erityisesti suun limakalvoilla.
Äidinmaito sisältää useita eri immunoglobuliineja (Ig), mutta sekretorinen IgA (sIgA) on suurin äidinmaidossa ilmenevä vasta-aineryhmä. Serologiset tutkimukset ovat osoittaneet, että HPV vasta-aineet ovat erittäin pitkäikäisiä, joten vaikka äidillä ei olisi aktiivista HPV-infektiota, voi äidin syljestä, seerumista tai maidosta löytyä HPV-vasta-aineita. Työ on aloitettu optimoimalla aikaisemmin syljelle käytettyä ELISA-määritystä (eng. **e**nzyme-**l**inked **i**mmunosorbent **a**ssay) äidinmaidon totaali IgA määrän mittaamiseksi. Tulokset äidinmaidon totaali IgA määristä olivat vahvasti yksilöriippuvaisia, mutta keskimäärin äidinmaito sisälsi IgA:ta noin 500 µg/ml (vaihteluväli 100–1000 µg/ml). Totaali IgA määritysten jälkeen lähdimme pystyttämään ja optimoimaan HPV16 spesifistä ELISA-määritystä. Työssä analysoitavat äidinmaitonäytteet (n=101) ovat osa TY:n Hammaslääketieteen laitoksen ja TYKS:n Naistentautien yhteistä HPV-infektion luonnollista taudinkulkua selvittävää pitkittäistutkimusta.

Asiasanat: ELISA, HPV16, IgA, papilloomavirus, totaali IgA, äidinmaito

Selection and biophysical characterization of DARPins

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In the past decades antibodies, including antibody fragments, have been a real success story treating a variety of diseases. More recently, however, direction has shifted towards multispecific therapeutics whereby multiple binding modules are combined onto a single molecule. These binding modules can consist not only of antibody fragments but also alternatives such as nanobodies and designed ankyrin repeat proteins (DARPins).

DARPins are single chain proteins that have been designed to bind specifically and in a similar manner to antibodies. The benefit of DARPins as therapeutics lies in their small size, 15 kDa, and lack of disulfide bonds and glycosylations. Importantly, their use in a multispecific context is attractive as DARPins are single chain molecules and as such are easily tethered together.

In this project DARPins were selected from a synthetically designed and constructed phage library using the phage display method. Selection of DARPins was performed against mCherry coupled to Avi-, ALFA-, twinstrep- and DoubleHis8 tags. Selections against mCherry were completed in four rounds yielding multiple binders specifically reactive to mCherry antigen. Next, selection was directed towards the histidine tag to see if it was possible to also find DARPins able to bind peptides. The DARPin binding surface is planar and not optimized for peptide binding, therefore the failure to find DARPins binding histidine tag was not unexpected.

In the second part of this project, 96 of the selected binding DARPins were sequenced. Sequences of interest, especially those that were repeated multiple times, were cloned, expressed and purified. Reference DARPins, anti-Her2, anti-EGFR and anti-serum albumin, were also expressed and purified to compare yields and characteristics such as aggregation propensity and thermostability as well as affinities. 10 ml culture yields of reference DARPins varied between 1,03-3,45 mg compared to selected library DARPins ranging from 0,13-1,44 mg.

Finally, tethering of two DARPins together to see impacts on biophysical behavior is ongoing. In this experiment, the hypothesis is the DARPin with the poorest biophysical properties determines the overall biophysical character of the tandem DARPins. Since DARPins in general are very stable and well behaving proteins, expectations are high for positive biophysical characterization.

Keywords: DARPin, phage display, multispecific therapeutics, mCherry, affinity

Ihmisen maitorauhasepiteelin haaroittumisrakenteen kuvantaminen ja kvantitointi

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Maitorauhanen on poikkeuksellinen elin, sillä sen rakenteessa tapahtuu suuria muutoksia koko eliniän ajan. Tämä normaali muuntelu tekee rintakudoksen alttiiksi syövän kehittymiselle. Useimmiten rintasyöpä saa alkunsa ihmisen maitorauhasen TDLU-rakenteesta (Terminal duct lobular unit). Tästä huolimatta, maitorauhasen kolmiulotteinen rakenne tunnetaan melko huonosti, sillä rauhanen on paksun rasvakudoksen ympäröimä, mikä rajoittaa kuvantamista. Uudet kudoskirkastusmenetelmät ja tehokkaampi mikroskopia ovat kuitenkin mahdollistaneet suurempien kudospalojen immunoleimauksen ja kuvantamisen kolmiulotteisessa muodossa.
Tutkimuksen tavoitteena oli selvittää immunoleimatun ihmisen maitorauhasen TDLU-rakenteen sisäistä haaroittumista kuvantamalla rakenteita kolmiulotteiseen muotoon light sheet -mikroskoopilla. Kolmiulotteista kuvantamista varten tutkimuksessa vertailtiin CUBIC- ja FUnGI-kudoskirkastusmenelmiä sekä optimoitiin FUnGI-menetelmää ihmisen maitorauhaskudokselle sopivaksi. Tutkittava kudosmateriaali saatiin rintojen pienennysleikkauksista sekä potilailta, joilla oli diagnosoitu rintasyövän esiaste (duktaalinen karsinooma *in situ*). Tutkimuksen tarkoituksena oli pyrkiä luomaan kolmiulotteisten TDLU-rakenteiden haaroittumista ja mittasuhteita kuvaava malli light sheet -mikroskopian pohjalta hyödyntämällä koneoppimista ja kuva-analyysiohjelmia.
Tutkimuksessa saatiin selville, että myrkytön ja nopeampi FUnGI-menetelmä kykeni kirkastamaan TDLU-rakenteet riittävän tarkasti, jotta kolmiulotteinen kuvantaminen oli mahdollista. Vaikka CUBIC-menetelmä kykeni kirkastamaan maitorauhasen kudosta tehokkaammin, sen tiedetään kutistavan kudosta vääristäen sen mittasuhteita, eikä se siten ole yhteensopiva TDLU-rakenteen mittaamisen kanssa. Vertaamalla *in vitro* -kasvatettuja ihmisen primääristen maitorauhasorganoidien haaroittumisrakenteita light sheet -kuvattuihin ihmisen TDLU-rakenteisiin havaittiin, että organoidien haarat vastaavat hyvin pitkälti oikean kudoksen haarojen mittasuhteita. Tulevaisuudessa tarkemman kolmiulotteisen TDLU-rakenteen ymmärtäminen voi hyödyttää myös rintasyöpätutkimusta.

Avainsanat: Maitorauhanen, TDLU, rintasyöpä, FUnGI-kudoskirkastus,
light sheet- mikroskopia


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Development of a novel affinity maturation strategy utilizing error-prone PCR and mammalian display

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In vitro affinity maturation is a strategy in antibody engineering where mutations are introduced into an antibody gene to improve its biophysical properties. Properties that are desired for an antibody can include high affinity and high thermal stability as well as low polyreactivity and low aggregation tendency. One strategy for the generation of an antibody affinity maturation library is to randomly mutagenize an antibody by error-prone PCR. The variants carrying favorable mutations can be subsequently isolated using an antibody display system. A powerful *in vitro* technology for antibody expression and isolation is mammalian display platform that allows the screening of millions of different whole IgG antibodies and the selection of biophysically favorable variants based on the antibody display level.

An approach for generating random mutations in either variable domain, variable light chain (V_L) or variable heavy chain (V_H), of an anti-gelsolin antibody and selecting favorable variants using mammalian display was tested to study alternative methods for affinity maturation. The mutagenized antibody library was stably transfected into the mammalian display system with around 4×10^5 and 3×10^5 variants with mutated V_L and V_H, respectively. Cells displaying the antibody at a high level were first enriched using magnetic activated cell sorting and subsequently sorted for the highest display level and antigen binding level using fluorescence activated cell sorter. The cells sorted for favorable mutations were 0.32 % and 0.43 % of the mutated V_L and V_H libraries, respectively.

According to the preliminary results, mammalian display seems to be capable of selecting biophysically favorable antibodies from a random mutagenesis library generated using error-prone PCR. Variants with improved display level and antigen binding level, compared to the parental antibody, will be characterized for biophysical properties and the types of mutations in cells displaying the antibodies at varying levels will be analyzed using next generation sequencing data acquired during the selection process.

Keywords: affinity maturation, antibody library, biophysical characterization, error-prone PCR, mammalian display

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Development of antibodies for non-competitive detection of saxitoxin

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Saxitoxin (stx) is a highly potent, heat-stable, prevalent natural alkaloid in aquatic environments produced by certain dinoflagellates and cyanobacteria. It is a small molecule with a molecular weight of 299.29 Da and it accumulates in aquatic ecosystem mainly in shellfish and clams. Consumption of stx-contaminated shellfish and clams may lead to food poisoning with variety of neural effects including convulsions and muscle and respiratory paralysis. Prevalence of stx is expected to increase in the future due to climate change and eutrophication. Thus, there is a need for an easy-to-use and reliable assay, with which the consumption and recreational use of saxitoxin contaminated water, and thereby food as well, can be prevented.

Current immunoassays for stx detection rely on reagent limited, competitive immunoassays. These kinds of immunoassays suffer from the problem that the signal with a low-concentration analyte is close to indistinguishable from zero. The hindrance for developing non-competitive, sandwich type assays for stx is the limited steric surface area, which prevents the simultaneous binding of two antibodies (Ab). For that reason, this research project utilizes anti-immunocomplex (anti-IC) Abs against a primary Ab bound to stx. The anti-IC Abs are to be found through phage display technology from a synthetic antibody library. So far, the focus of the work has been on cloning and producing two different primary Abs, 1E8-Fab and 5F7-Fab in *E. coli* XL1-Blue electrocompetent cells and testing their specificity on stx with an immunoassay utilizing stx-HRP conjugate.

The 1E8 shows over 50 times higher specific signal on stx than 5F7, and thus binds stx better than 5F7. The expression yield of the Fabs was also tested which turned out to be low, maximum 0.13 mg/L in cell at and 0.25 mg/L in culture medium after 24h expression. The better yield was with 5F7 which has the worse specificity on stx.

Due to low yield of Fabs, the expression is next tried with different cell strains, *E.coli* BL21 and RV308, and likely with mammalian cells as well. When sufficient yield is reached, the sensitivity of the two capture-antibodies will be tested.

Keywords: saxitoxin, cyanotoxin, non-competitive, immunometric, immunoassay, anti-immunocomplex

Development of α-defensin lateral flow test for prosthetic joint infections

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Prosthetic joint infection (PJI) is disastrous complication and is the most common reason for revision after a primary total hip arthroplasty. The diagnosis is of PJI is challenging due to it not having clear clinical signs or biomarkers for detection and is currently diagnosed with evidence-based diagnostics of one major or two minor criteria. The highest weighting minor criterium is positive α-defensin in synovial fluid of the joint.

α-defensins are antimicrobial peptides expressed in neutrophils and intestinal Paneth cells that upon interaction with infectious pathogens exert antimicrobial activity. Human α-defensins include four neutrophil-derived α-defensins, human neutrophil peptide 1-4 (HNP1-4) of which HNP1-3 have close similarity. The detection of synovial α-defensin can be performed via an ELISA or with a Synovasure -lateral flow test both of which are either too time consuming or costly. Ultimate aim of this study is to develop a quantitative lateral flow approach for α-defensin detection. Recombinant α-defensins are expressed in an *Escherichia coli* expression system. Produced peptides are used to screen for α-defensin recognizing antibodies which will be used to for a sandwich immunoassay. Tracer antibodies are conjugated with Upconverting nanoparticles (UCNP) which's fluorescent signal can be measured with a reader for easy quantitation. The test is optimized with isolated human neutrophils and ran with cohorts of patient samples. HNP1, HNP3 and HNP4 peptides were produced in *E. coli* T7 NEB express cells for their durability over BL21(DE3). Due to HNPs antimicrobial nature the peptides were expressed with a cleavable propeptide with a polyhistidine-tag (HisTag) and therefore did not kill the cells. Accidentally, the used vector backbone pET28a+ had an additional HisTag that was expressed with our peptide presumably increasing affinity in purification. Purification is still in development, but purified peptides are used to screen for binders from a Fab-library. Found binder-pairings are optimized using Human α-defensin 1 ELISA -kit as a reference for sensitivity and specificity. Ultimately, the test is expected to recognize at least one form of human α-defensin and is implemented onto a lateral flow platform.

Keywords: α-defensin, antimicrobial peptide, biomarker, diagnostics, HNP1-4

Optimizing pre- and post-crosslinking methods for alginate using the Triaxial tool print head with microfluidic chips or mist sprayer

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Generally, 3D bioprinting is used as a layer-by-layer method to create tissue-like structures. Alginate is a widely used bio-ink in the biomedical field, including tissue engineering and drug delivery due to its biocompatibility, biodegradability, and non-toxicity. One of the advantages of using alginate is the possibility of crosslinking by calcium chloride (CaCl2). The main idea of crosslinking is to solidify the bio-ink into a stable structure. The project was done in a Turku-based bioprinting company called Brinter Oy. The primary aim of this master's thesis was to design and test resin-based microfluidics to develop the mixing of alginate using Brinter's Triaxial tool print head. Additionally, different pre- and post-crosslinking methods of alginate, such as spraying using CaCl2, were compared and analyzed.

The preparation of microfluidics consisted of designing, printing, curing, and optimizing the model of the different fluidic chips. Resin-based microfluidics, which combined isopropyl alcohol (IPA) washing and UV curing, enabled high-resolution printing. Alginate bio-inks were prepared and dyed visible by using different food colourings. The mixing of the bio-inks was analyzed using the RGB values as a correlation. The parameters were measured using the Adobe Capture application. The effect of microfluidics on the reproducibility of printing was tested using different-sized printed objects. Moreover, the post-crosslinking method of alginate by spraying CaCl2 during the printing was compared to other crosslinking methods.

The results of this project emphasize the importance of using microfluidics in 3D bioprinting in the future. Achieved RGB values indicate a good mixing of different coloured materials. The multifunctionality of fluidic chips enables many applications, such as material mixing and crosslinking during printing. One of the possible modifications of spraying could be creating an aseptic environment with specific conditions inside the printing hood during the bioprinting.

Keywords: 3D bioprinting, microfluidics, alginate, crosslinking, spraying

HALUAMME SINUN TYÖSKENTELEVÄN MIELEKKÄISSÄ JA KOULUTUSTA VASTAAVISSA TEHTÄVISSÄ

Loimu haluaa jäsenistönsä työskentelevän mielekkäissä ja koulutusta vastaavissa tehtävissä. Siksi tarjoamme jäsenillemme laadukkaita urapalveluita, joiden avulla mielekkään työn metsästys on helpompaa!

Tuemme jäseniämme monipuolisesti työnhaun ja työuran eri vaiheissa. Olitpa sitten aloittamassa työnhakua ja pohtimassa omaa osaamistasi, jo hyvässä vauhdissa hakemustesi kanssa, menossa työhaastatteluun tai jo työssä, urapalveluistamme on varmasti hyötyä juuri sinulle!

Tarjoamme jäsenillemme esimerkiksi seuraavia urapalveluita:

- Henkilökohtainen työnhaku-, ura- ja palkkaneuvonta
- Monipuoliset koulutukset ja webinaarit työnhakuun sekä uralla etenemiseen
- Mahdollisuuden osallistua mentorointiohjelmaan, jossa pohditaan osallistujien uramahdollisuuksia ja vahvuuksia
- Potentiaalisten työnantajien listausten laatiminen
- Oman osaamisen tunnistaminen ja sanoittaminen

Tarjoamme jäsenillemme laadukkaita urapalveluita

LOIMU

Detection and quantification of methionine 1-linked ubiquitin chains in intestinal inflammation and cancer models

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Ubiquitination is a post-translational modification which involves the attachment of polyubiquitin chains to substrate proteins. Methionine 1-linked ubiquitin chains (Met1-Ub) have important roles in nuclear factor-κB transcription factor activation in immunity and inflammation. To study Met1-ubiquitination in intestinal inflammation and carcinogenesis, this study aimed to produce an anti-M1 antibody and a Met1-specific ubiquitin binder (Met1-SUB) and to apply them for Met1-Ub detection in Western blot, immunocytochemistry, and immunohistochemistry.
Anti-Met1 antibody and Met1-SUB were produced recombinantly in mammalian and bacterial cells, respectively. Their function and specificity were tested in Western blot with colorectal and prostate cancer cells and mouse colon tissue. The antibody was used in immunocytochemistry to stain and quantify Met1-Ub in cancer cells, and optimized for immunohistochemistry staining to study Met1-ubiquitination in the colon of keratin 8 knockout ($K8^{-/-}$) mice, a chronic colitis model.
The antibody was able to specifically recognize changes in Met1-Ub expression in cell and tissue lysates after immunoprecipitation with Met1-SUB. Immunocytochemistry staining showed significant differences in Met1-ubiquitination upon overexpression or inhibition of Met1-Ub. However, the antibody could not detect changes in Met1-Ub expression in wildtype and $K8^{-/-}$ mice colon. This study provides versatile tools for Met1-Ub detection that upon further optimization could be applied to other model systems as well, including tumor organoids and the fruit fly. In the future, these tools could potentially be used to study Met1-ubiquitination in patient samples and aid in the discovery of new biomarkers for inflammatory bowel disease and colorectal cancer diagnostics.

Keywords: Met1-linked ubiquitin, inflammation, antibody, ubiquitin binder, Western blot, immunocytochemistry, immunohistochemistry, inflammatory bowel disease, cancer

The role of Hippo regulators on Neuropathic pain

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Neuropathic pain is a major clinical challenge owing to its physiological elusiveness and lack of substantially efficient pharmacotherapies. Previously, it has been demonstrated that the Hippo pathway can be a trigger for the development of neuropathic pain, yet the mechanistic procedure of how this is achieved at the molecular level remains poorly understood. Recent studies have shed the light on Hippo-regulators, Ror2 and Wnt5A which are part of the non-canonical Wnt signalling, to be dysregulated in different models of pain.
To achieve a molecular understanding of Ror2 and Wnt5A in neuropathy, expression studies at the genomic and proteomic levels were performed on the different components of the nervous system including dorsal root ganglia (DRGs), Spinal cord (Sp), brain and sciatic nerve (Sc) using the chronic constriction injury (CCI) pain model. The functional role of Wnt5A in enhancing neural excitability was explored by measuring intracellular calcium levels in dissociated sensory neurons. IMR-32 human neuroblastoma cell line was validated for future target studies by analyzing the expression profile of our targets.
Herein, the expression of our targets was confirmed in the relevant tissues of the pain pathway. Ror2 immunoreactivity was found in the cell bodies of DRGs and the axonal terminal. We report increased Ror2 and Wnt5A levels in the Sc and DRGs, respectively. We also observed that Wnt5A enhances the excitation of the sensory neurons. Finally, we were able to identify the IMR-32 cells as a suitable in vitro model for future studies.

Keywords: neuropathic pain, Ror2, Wnt5a, CCI, IMR-32 cells, DRG, sciatic nerve

Development of blood and saliva sample preparation workflows for isothermal POC MDx assays

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Human cytomegalovirus (CMV) is the leading cause of congenital viral infections in children and the leading non-genetic cause of sensorineural hearing loss. In addition, CMV infection is a significant cause of neurodevelopmental disabilities leading to neonate morbidity and even occasional mortality. Despite the high prevalence and pathogenicity of CMV, standardized approaches and diagnostic tests are lacking for large-scale screening. Loop-mediated isothermal amplification (LAMP) is an emerging amplification technique for nucleic acids, and it has potential applications in point-of-care (POC) diagnostics with high sensitivity and specificity. The aim of this master's thesis work is to develop sample preparation workflows with various sample types for isothermal point-of-care assays.

The assay was conducted with loop-mediated isothermal amplification. The reaction was optimized with six different master mixes, $MgSO_4$ concentration optimization, four LAMP primer sets, and temperature gradient. Sample preparation workflows for CMV spiked blood, urine, and saliva samples were optimized with various incubation conditions, sample dilutions, and red blood cell –depletion protocols. LAMP assay was utilized with the thermal incubation and fluorescent measurement properties of quantitative PCR (qPCR) instrument. In addition, the final version of the assay was constructed with dry LAMP reagents and experimental POC –arrangement.

Limit of detection was determined to be 2000 IU/ml for blood, 1500 IU/ml for saliva, and 1000 IU/ml for urine. This study demonstrates that a straightforward POC assay with isothermal amplification is suitable for CMV testing. Isothermal nucleic acid amplification is promising with difficult sample materials, such as blood and saliva, which are often challenging sample materials for qPCR assays due to inhibitors. In addition, thermocycling is not needed for LAMP workflow, and therefore the requirement for expensive and complex equipment is eliminated. These results will aid the future applicability and design of POC –methods for CMV and other target analytes. With the help of study discoveries, it is easier to assess the applicability of the POC assay possibility for CMV diagnostics.

Keywords: human cytomegalovirus, sample preparation, loop-mediated isothermal amplification, point-of-care, nucleic acid amplification

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Establishment, characterization, and validation of dorsal root ganglia explant cultures for drug development

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

While pain is an unpleasant sensation it normally has important function protecting the body against potential harm. Chronic pain on the other hand is maladaptive and pathological disease and no longer beneficial. Dorsal root ganglia (DRG) which contain cell bodies of sensory neurons convey nociceptive information about internal and external stimuli. DRG *ex vivo* explants preserve the tissue-like microenvironment making them appropriate models to study neuronal functions for drug testing and development. The aim of the project is to establish an *ex vivo* rat DRG explant model to study pain-related phenotypes, characterize the cellular composition, validate the model by studying nerve growth factor (NGF) dependence and use viral vectors to express foreign genes in DRG.

Rat DRG explants were grown *ex vivo* in a culture medium with and without NGF employing Matrigel at different concentrations. Unbiased automated live cell imaging was performed with Incucyte and neurite outgrowth was quantified utilizing Incucyte's NeuroTrack software and Qupath. The developed protocol was applied to track neurite growth in the presence of tool compounds, such as NGF and tropomyosin receptor kinase A (TrkA) antagonist *in vitro*. Cell populations were immunostained with specific neuronal and glial markers. Explants were also transduced with adeno-associated virus (AAV) vector for enhanced green fluorescent protein expression.

In this project, I successfully established protocols for dissection, culturing, and morphological analysis of rat DRG explants. Results indicate that neurite growth is proportional to Matrigel concentration; being highest at 100% and smallest at 10%. Growth of DRG explants is also age-dependent; explants from 3-7 weeks-old rats have higher probability to grow (80-96%) than explants from 9-weeks-old rats (48%). Immunostaining confirmed a close association between neuronal and glial cells at newly formed neurites. I developed a working quantification protocol for neurite growth to show an increase in sprouting in the presence of NGF during the first six days, suggesting a temporal dependence on NGF. TrkA antagonist in concentrations of 300 nM and 1 µM showed inhibited sprouting with and without NGF. Recombinant AAV vectors successfully transduced cells in DRG suggesting them being powerful tools to transfer e.g., pain related therapeutic genes.

Keywords: chronic pain, dorsal root ganglia, explant, explant culture, nerve growth factor, tropomyosin receptor kinase A


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Development of lateral flow assay system with magnetic particles as solid support

Oskari Nääjärvi
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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

The test line in traditional lateral flow immunoassay (LFIA) is formed by immobilising capture antibodies onto a nitrocellulose membrane. Such lateral flow immunoassays are widely used, but despite of their possibilities, systems rely on stationary test line.
The aim of this research is to increase sensitivity by developing lateral flow immunoassay and sample preparation workflow by immobilizing capture antibodies onto spherical magnetic nanoparticles, which are collected to form a test line by an external magnetic field. Currently magnetic particles in lateral flow systems have been mainly used as assay reporters. The use of magnetic particles as a solid support in LFIA is a novel approach.
Surface area for immunocomplex formation is larger than in conventional LFIA due to spherical surface of magnetic nanoparticle, immunocomplex formation time is increased, and background signal can be reduced with heavier washing, as the immunocomplex is held by magnetic force.
Magnetic nanoparticles (80, 250, 500 and 1000 nm) and upconverting nanoparticles (25 nm) were coated with streptavidin to test components and functionality of assay, using biotinylated bovine serum albumin as linker molecule. The flow of particles was tested on several nitrocelluloses. Suitable test cassette was 3D printed and different arrangements of magnets were also investigated.
Optimal flow on NC was achieved with 250 nm MNP's and lowest background signal with upconverting nanoparticles with 5 mM EDC ((1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) treatment and Whatman FF80HP Plus nitrocellulose. Currently test cassettes which can stop MNP's as the test line are already developed. Once the whole streptavidin-biotin complex is measurable, the aim is proof-of-concept immunoassay for thyroid-stimulating hormone.

Keywords: lateral flow immunoassay, magnetic nanoparticles, magnetic field, 3D-printing, diagnostics

Selection platform for biparatopic DARPin-antibody fusion molecules

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Whole antibodies and antibody fragments (such as fragment antibody binding, Fab and single-chain fragment variable, scFv) are commonly used as affinity reagents and as therapeutics. The process of generating novel antibodies is also widely researched and there are many established techniques for the library generation, selection, and validation processes. However, sometimes the selected antibodies might have some limitations, such as suboptimal affinity towards the antigen.
Designed ankyrin repeat proteins (DARPins) are a class of protein scaffolds that can be enriched and selected against any target antigen in nano- and picomolar affinities. They are highly stable 14-21 kDa proteins consisting of usually two to six repeats, each 33 amino acids long, which form a hydrophobic core and continuous binding surface. During the last decade DARPins have been the most researched class of alternative scaffolds for therapeutics as well as other applications.
The aim of this thesis was to develop a selection platform for biparatopic DARPin-antibody fusions to boost affinity of existing antibodies. Using a randomized synthetic DARPin library, binders were enriched against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleoprotein N. The enriched binder pools were then attached to a nucleoprotein N specific antibody RS16D01 with PCR and type IIs restriction enzymes. The Fab- and scFv-DARPin fusions were designed to include a flexible $(GGGGS)_n$-linker in between the two molecules (n = 1, 2, 4 or 6). DARPins were attached to both N- and C-terminus of scFv, and only to N-terminus of Fab. After further enriching the fusion molecules, the pool of selected clones was screened as phages. Multiple potential clones that expressed various different linker lengths were found in further characterization and sequencing. For example clone JT007H02 had 8.2-fold higher signal to background ratio compared to the parental when 1.1E8 cfu phages were used in a phage immunoassay, which could indicate enhanced binding properties towards nucleoprotein N.
This proof-of-concept study demonstrates that functional biparatopic DARPin-RS16D01 fusion molecules can successfully be created. Thus, attaching enriched DARPins to existing parental antibodies via simple, flexible $(GGGGS)_n$-linkers, could be an alternative and effective approach to boosting antibody affinities.

Keywords: designed ankyrin repeat proteins, DARPins, biparatopic, fusion molecules, antibodies

Novel antibody library design: Super stable disulfide bridge stabilized single chain variable fragment antibody library

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

The smallest antibody fragment that can bind to an antigen is the single chain variable fragment (scFv). Its size can be advantageous in many biotechnological and clinical applications. However, compared to other antibody fragments, it has lower thermostability. One of the methods to improve its stability is to introduce an extra disulfide bond between two complementary determining regions (CDR-H3 and L1).

This work aims to generate a novel single-chain variable fragment (scFv) antibody library with an unnatural disulfide bond enhancing the stability of the single-chain variable fragment. The framework for the library is Trastuzumab, a commercial antibody against human epidermal growth factor receptor 2. Although one of the intradomain disulfide bonds were omitted to facilitate phage display as its existence raise expression issues in *E.coli*.

To pursue this, the CDR-L1 and L3 were randomized by degenerate oligos. The resulting library was panned against protein L to eliminate clones with frameshifts and improper folding. The CDR-H1 and H2 were amplified from an existing functionally selected fragment antigen binding library. The diversification of the CDR-H3 will be done by Trim Oligos in five different loop lengths of 13, 14, 15, 17, and 19 amino acids. All fragments will be combined through simultaneous LguI digestion and ligation for seamless cloning. The resulting fragment will be cloned in pEB3v3 and by the transformation in *E. coli* SS320 the final library will be generated.

Light chain library size after transformation was $7.52x10^7$ which covers $6.53x10^3$ times the diversity design. Considering the diversity design of the primers, in theory, the final library diversity would be $5.32x10^{28}$. After protein L selection, 10 clones were sequenced that all were in frame. However, due to practical limitation this diversity is not achievable. After replacing the missing intradomain disulfide bridge, it can be used for development of superstable scFv fragments for various targets.

Keywords: antibody library, CDR, disulfide-stabilized scFv, diversification, phage display

Affinity maturation of anti-gelsolin antibodies

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Gelsolin amyloidosis is a hereditary aggregation disease caused by a point mutation. The change in amino acid composition affects the binding of Ca^{2+} to the protein leading to aberrant proteolytic cleavage, which eventually leads to the formation of the 8 kDa and 5 kDa gelsolin fragments. The fragments, mostly the 8 kDa fragment, form aggregates, which causes the symptoms of the disease.
In this thesis, affinities with improved affinity to the 8 kDa gelsolin fragment were developed from the previously developed CDRH3 and CDRL3 Fab libraries from parental Fab OHA5 with phage display. After enrichment with one panning round, the enriched light and heavy chain mutations were joined together to a combinatorial library, followed by further enrichment with two more panning rounds. The enriched binders were then primary and secondary screened in immunoassays and sequenced. Based on the secondary screening results ten binders were converted to immunoglobulins and expressed in mammalian cells.
The Fabs chosen for IgG conversion had three-to-seven-fold increase in signal in the secondary screening immunoassay. These preliminary results suggest that the affinity has been improved in the ten Fabs chosen for further studies.
The sequenced variants had mutations only in amino acid position 125 in the CDRH3, suggesting that the other positions were already mature. In the CDRL3, amino acid position 113 was mutated on all but one sequenced variant. Additionally, all the mutated positions had mutations. The next step of this study is to study the biophysical properties and affinities to the 8 kDa gelsolin fragment of these selected binders in immunoglobulin format.

Keywords: affinity maturation, antibody engineering, gelsolin amyloidosis, phage display, therapeutic antibodies

Generation of clinical diagnostic antibodies against common lymphatic endothelial and vascular endothelial receptor 1

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Common lymphatic endothelial and vascular endothelial receptor 1 (CLEVER-1) is a scavenger glycoprotein receptor expressed on the surface of a subset of immunosuppressive macrophages. CLEVER-1 promotes tumor growth and spread in cancer. Immunotherapeutic blockade of CLEVER-1 delays tumor growth by activating cytotoxic CD8+ T-cells. Bexmarilimab, a novel anti-Clever-1 antibody, has shown promising efficacy and safety in phase II clinical trials. This study aims to optimize bexmarilimab dosing by developing a companion diagnostic quantitative time-resolved fluorescence immunoassay (TRFIA) for CLEVER1 using novel antibody fragments.

The University of Turku Fab phage display libraries were panned against the in-house generated recombinant CLEVER-1 protein. The epitopes of the newly discovered Fabs were mapped to recombinant human CLEVER-1 protein using TRFIA, to confirm their non-competitive binding to bexmarilimab epitope. The best antibody fragments were sequenced and affinity-measured with bilayer interferometry. They were then used for validation in a TRFIA to measure the concentration and receptor occupancy of CLEVER-1 protein in human plasma.

After three rounds of Fab phage library panning, considerable enrichment of the libraries was observed with a specific CLEVER1 binding to background ratio of 85.61 and final enriched library output of 3.54E12 CFU/mL. Followed by screening for the best Fabs, eight clones demonstrated over 150 signal to background ratio in specific binding to CLEVER1. After sequencing, the fragments were identified as having different CDR regions. Bexmarilimab epitope blocking did not significantly affect binding of the Fabs to CLEVER1, with an average Fab binding decrease of 9% (P=0.2267) The affinity of the best Fabs as K_D values ranged from 1.41 nM to 16.5 nM. The results from the final validation TRFIA on healthy serum samples were in line with the CLEVER1 normal range in serum, which is 20-40 ng/mL. CLEVER1-depleted serum was used as negative control.

To conclude, six unique Fab antibody fragments were selected against CLEVER1, which bound to the desired epitopes. Using the novel fragments, a TRFIA was developed to measure the CLEVER1 concentration and bexmarilimab occupancy.

Keywords: bexmarilimab, cancer, CLEVER1, phage display, TRFIA

Isolation of rhinovirus-specific Fab antibodies from phage display antibody library

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTIC

Human rhino viral (HRV) diseases continue to be underdiagnosed, despite their negative impact on society, which includes not only exacerbation of underlying respiratory diseases in immunocompromised people and elderly people, but also missed working hours and increased healthcare costs. Besides nucleic acid-based methods, there are no antibodies available for immunodiagnostic purposes due to high antigenic variation of over 150 different HRV serotypes.
The study aimed to discover recombinant antibodies with the capacity to recognize a broad range of different HRV serotypes for the development of a generic HRV immunoassay. More specifically, the objective was to generate binders specific for a conserved region of HRV 3C protease by selecting them from universal fragment-antigen-binding (Fab) phage display libraries.
Two different selection strategies were tested. First, the biopanning was performed by switching between the antigens of HRV 3C protease and a 3C-peptide derived from the selected conserved region of the protein. In the second approach, the gene coding for the HRV 3C protein was mutated at a specific 3C peptide site and the mutated version of the protein was then used as a soluble blocker in the biopanning reaction against the HRV 3C protein. Selected phages after three panning rounds were analyzed in the phage immunoassay. Enriched libraries from panning were cloned into a screening vector to express soluble Fabs in E. coli, and eventually, in total, 976 Fab clones were analyzed in sandwich immunoassay. Eighty-six clones that gave signal for the target protein and/or the peptide were further analyzed in secondary screening and direct sequencing.
The phage immunoassay data confirmed a good enrichment, and screening and sequencing data revealed several different specific binders. Ten binders that gave the highest signals for both the protein and the peptide were selected from the first panning approach. The selected Fabs were then expressed in bigger culture volumes, purified and used for kinetic measurements. The Fabs will be tested on viral infected cell lines using immunofluorescence microscopy. Some promising Fabs recognize the desired target epitope and are interesting candidates for further development into a diagnostic assay for HRV.

Keywords: human rhinovirus, 3C protease, phage display, Fab, recombinant antibody library

Anti-EPLIN antibody development

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Head and neck cancers are the seventh most prevalent cancers worldwide with the predominant subtype head and neck squamous cell carcinomas (HNSCC) accounting for over 90 % of these cases. Late diagnosis and start of treatment, insufficient prognosis, and high mortality rates due to lack of biomarkers and screening methods for HNSCCs have presented an issue in treatment of the patients.

Epithelial protein lost in neoplasm (EPLIN) is a cytoskeletal actin binding protein expressed as two isoforms, EPLIN-α and EPLIN-β. According to still unpublished studies, EPLIN-α protein has been linked with aggressive clinical behaviour of HNSCC. Currently, there are commercial antibodies detecting total EPLIN, however no available binders are capable of specifically recognizing just either one of the isoforms. The objective of this MSc' thesis project was to develop an antibody for the detection of EPLIN-β isoform.

The project was carried out by generating a rediversified antibody binding fragment (Fab) library by shuffling variable heavy (V_H) domains of previously enriched single chain fragment variable (scFv) binders and variable light (V_L) domains of a universal phage display library. The Fab binders were then enriched by phage display method in three rounds of panning against a peptide of EPLIN-β followed by screening for high-affinity binders with immunoassays as well as sequencing of positive clones. Additionally, seven clones were characterized by half maximal effective concentration (EC_{50}) determination.

Sequencing of the seven Fab clones showed each of them having a unique V_L chain whereas for V_H three different sequences were found. The most common V_H chain appeared in four clones showing good binding capabilities with EC_{50} values of 10-13 nM. After conversion to intact IgG, these binders can be in immunohistochemistry application for prognosis of HNSCC.

Keywords: head and neck squamous cell carcinoma, HNSCC, phage display, antibody, epithelial protein lost in neoplasm, EPLIN

TRIOLAB

Triolabilta tuotteet tutkimuksen tehostamiseen

Optinen genomikartoitus

Optinen genomikartoitus on täysin uudenlainen tapa analysoida genomin suuria rakenteellisia variaatioita. ***Bionanon Saphyr*** automatisoi optisen genomikartoituksen. Automatisoitu työnkulku vähentää mekaanista käsityötä ja tulosten tulkinnan variaatiota.

bionano

Sovelluksia mm.

- diagnosoimattomat geneettiset sairaudet
- hematologiset syövät
- geenitutkimus ja geeniterapia
- solulinjojen stabiilisuustutkimukset
- syöpäkasvainten tutkimus
- geenitekniikan tutkimukset
- evoluutiobiologian tutkimukset
- referenssigenomien kokoaminen

Monileimalukijat

Tecanin Spark-monileimalukija soveltuu ELISA-mittauksista ja perustutkimuksesta aina kuvantamiseen ja vaativaankin lääkeaineherkkyystestaukseen. ***Spark Cyto -kuvantavalla monileimalukijalla*** voidaan analysoida soluja fluorisenssikameran avulla kuoppakohtaisesti koko tutkimuksen ajan.

TECAN

Sovelluksia mm.

- genomiikka ja proteomiikka
- mikrobiologia
- solututkimus
- lääkeainetutkimus
- elävien solujen kuvantaminen
- automatisoitu solujen laskeminen (myös viability)
- absorbanssi-, fluoresenssi- ja luminesenssianalyysit

Soita tai kirjoita ja kysy lisää meiltä. Voidaan myös verkostoitua Linkedinin kautta!

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Utilizing MALDI-TOF MS to distinguish clinically relevant strains of *C. diphtheriae*

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Diphtheria is a potentially fatal infection with a case mortality rate between 5-17%, mostly affecting children younger than 15 years and is especially dangerous for unimmunized or immunocompromized populations. Dipththeria is mainly caused by the bacterium *Corynebacterium diphtheriae,* (*C. diphtheriae*), which is generally divided into four biovars in descending level of severity: gravis, mitis, intermedius and belfanti.
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is not currently used to identify the different biovars of *C. diphtheria,* responsible for diphtheria of differing severity. This is because the resolution of the MALDI-TOF MS is insufficient to distinguish between their differences. The aim of the thesis work was to distinguish between biovars by utilizing differences in their metabolic pathways. First biovars gravis, mitis and intermedius were grown on a Bordet-Gengou blood agar to isolate intermidis. Gravis and mitis were suspended in a starch saturated water solution, to distinguish gravis based on its ability to ferment starch, using MALDI-TOF to analyze the products. The initial results were unsuccessful, but the overall technique would require further investigation to establish any feasibility with certainty.
However, it is diphtheria toxin, rather than *C. diphtheriae* itself, which causes severe diphtheria. Therefore, the ability to distinguish between diphtheria toxin positive and negative strains of *C. diphtherieae* would be of clinical value. To detect diphtheria toxin, using MALDI-TOF, it needs to be broken down into its subcomponents. Different breakdown treatments were attempted using mercaptoethanol, urea and sodium dodecyl sulfate respectively. Preliminary results showed that *C. diphtheriae* which had been treated with sodium dodecyl sulfate produced additional mass spectrum peaks in 8/12 cases which were not otherwise visible, indicating the successful breakdown of diphtheria toxin. However, more research across more *C. diphtheriae* strains would be needed to demonstrate the viability of this method with confidence.

Keywords: *C. diphtheriae*, diphtheria toxin, gravis, intermedius, MALDI-TOF, mitis

CA125 glykovarianttimäärityksen muuntaminen magneettipartikkelipohjaiseksi kemiluminesenssimääritykseksi

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Munasarjasyöpä on maailmanlaajuisesti naisten seitsemänneksi yleisin syöpä. Vuosittain todetaan noin 240 000 uutta tapausta ja taudin viiden vuoden eloonjäämisaste on noin 49 %. Munasarjasyöpään liittyy usein CA125-proteiinin lisääntynyt määrä verenkierrossa. Lisääntynyt CA125-pitoisuus liittyy kuitenkin myös muihin sairauksiin kuten endometrioosiin, mikä vaikeuttaa kyseisen analyytin käyttöä diagnostiikassa. Munasarjasyövässä usein myös CA125-proteiinin glykosylaatiossa tapahtuu muutoksia ja näitä syöpään liittyviä glykovariantteja voidaan hyödyntää biomarkkerina munasarjasyövän diagnostiikassa. Työn tarkoituksena oli muuntaa olemassa oleva aikaerotteista fluoresenssia hyödyntävä glykovariantin tunnistava immunomääritys magneettipartikkelipohjaiseksi kemiluminesenssimääritykseksi. Kemiluminesenssia mittaavat laitteet ovat yleisesti käytössä sairaalalaboratorioissa, jolloin täällä muutoksella voidaan tuottaa määritykselle lisää kaupallista arvoa.

Kehitettävässä määrityksessä käytettiin magneettipartikkeleita, jotka oli pinnoitettu glykovariantin tunnistavalla vasta-aineella. Nämä partikkelit kiinnittyivät analyytin glykosyloituun osaan. Määrityksessä käytettiin lisäksi HRP-entsyymillä leimattua CA125 proteiinin tunnistavaa vasta-ainetta. Signaalin tuottamiseen käytettiin kemiluminoivaa substraattia. Tällöin määritys tunnistaa vain glykovariantin eikä normaalia CA125-proteiinia. Työssä vertailtiin muun muassa eri puskurien ja magneettipartikkelien vaikutusta määritykseen. Kehitettyä määritystä varten olemassa olevaa laitteistoa piti muokata mm. 3D-tulostamalla 96-kuoppalevylle magneettiteline, joka oli yhteensopiva levypesurin kanssa.

Työssä havaittiin, että määrityksen suorituskykyyn vaikuttaa puskurien koostumus ja magneettipartikkelien ominaisuudet. Erityisesti magneettipartikkeleilla suurempi vasta-aineiden sitomiskapasiteetti paransi määritystä huomattavasti. Määritystä testattiin seerumi- ja potilaisnäytteillä. Näistä saatiin lupaavia tuloksia, mutta määritys vaatii edelleen optimointia, jotta se saadaan toimimaan luotettavasti potilasnäytteillä. Työssä onnistuttiin siis osittamaan määrityksen toimivuus konseptitasolla, mikä on lupaavaa määrityksen kehittämisen jatkamiseksi.

Asiasanat: CA125, glykovariantti, immunomääritys, kemiluminesenssi, magneettipartikkeli, munasarjasyöpä

Geno1 teknologian automatisointi syöpädiagnostiikassa

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Syöpään sairastuu vuosittain miljoonia ihmisiä. Taudin varhainen havaitseminen on onnistuneen hoidon kannalta ensiarvoisen tärkeää. Nestebiopsia mahdollistaa syöpämutaatioiden luotettavan tutkimisen potilaan verinäytteestä, jolloin näytteenotto on hyvin yksinkertaista ja potilaalle helppoa. Geno1 teknologian avulla veren soluvapaasta DNA:sta tutkitaan syöpämutaatioita sisältävät geenialueet. Geno1 teknologialla valmistetut DNA-kirjastot sekvensoidaan uuden sukupolven DNA sekvensointi alustoilla ja näytteissä olevat syöpämutaatiot etsitään Geno1 tietojenkäsittely algoritmien avulla.
Diplomityön tavoitteena oli automatisoida Geno1 teknologian koko laboratorio-prosessi, mukaan lukien magneettipartikkeleihin perustuva DNA-kirjastojen puhdistukset ja reagenssien esikäsittelyt. Automatisointi toteutettiin Opentronsin OT-2 robotilla, jota ohjattiin Python ohjelmointirajapinnan avulla.
Diplomityössä onnistuttiin koko Geno1 prosessin automatisoinnissa ja saadut tulokset olivat täysin vertailukelpoisia manuaaliseen prosessiin. Automatisointi vähentää ihmisen tekemää työmäärää, minimoi virheet sekä säästää aikaa. Geno1 prosessin automatisointi tekee teknologiasta entistä luotettavamman, toistettavamman ja kustannustehokkaamman.

Asiasanat: syöpädiagnostiikka, robotiikka, nestebiopsia, nukleiinihapot, polymeraasiketjureaktio, sekvensointi

HIDEX

Lateraalivirtausmääritys syöpämerkkiaineelle CA19-9 munasarjasyövän diagnostiikkaan

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Munasarjasyöpä on yksi tappavimmista gynekologisista sairauksista. Koska munasarjasyöpä on aluksi oireeton, se ehtii usein leviämisvaiheeseen ennen diagnoosia. Lisäksi diagnosoinnin apuna käytettävät syöpämerkkiaineet voivat olla koholla myös hyvänlaatuisissa tiloissa, kuten endometrioosissa. Syöpäsoluissa proteiineille tapahtuu glykosylaatioita, jotka ovat usein syöpäspesifisiä, ja näitä proteiinien glykovariantteja voidaan havaita jo syövän aikaisissa vaiheissa. Perinteisesti syöpämerkkiaineita havaitaan proteiiniepitooppeja tunnistavilla vasta-aineilla. Määritysten tarkkuutta eli syöpäspesifisyyttä on mahdollista parantaa käyttämällä glykosylaatioita tunnistavia vasta-aineita.

Hiilihydraattiantigeeni CA19-9 on merkkiaine, joka on koholla useissa syövissä, mutta myös esimerkiksi haimatulehduksessa ja kirroosissa. Tällä hetkellä sitä hyödynnetään lähinnä haimasyövän diagnosoinnissa, mutta se on potentiaalinen syöpämerkkiaine myös munasarjasyövän diagnosointiin. CA19-9 esiintyy usein kantajaproteiinien, esimerkiksi muiden syöpämerkkiaineiden kuten CA125, CA15-3 ja CEA pinnalla.

Työn tarkoituksena on kehittää munasarjasyövän merkkiainetta CA19-9 mittaava lateraalivirtaus-immunomääritys. Työssä on testattu useita eri määrityksiä, joista osa mittaa pelkkää CA19-9:n määrää, ja osa sen määrää eri kantajaproteiinien pinnalla. Tavoitteena on kehittää määritys joka mahdollistaa aikaisen diagnoosin, sekä erottelun hyvänlaatuisista tiloista ja rajatapauksista. Työssä on käytössä luminoivia leimoja perinteisten kolorimetristen leimojen sijaan. Käytettävien leimateknologioiden avulla määrityksestä on mahdollista saada herkkä ja kvantitatiivinen. Lisäksi lateraalivirtausformaatin ansioista määritys on myös helppokäyttöinen, nopea ja vieritestaukseen soveltuva.

Tällä hetkellä pelkkää CA19-9:ä tunnistava vasta-ainepari toimii ja erottelee positiivisen näytteen negatiivisesta. Kantajaproteiinien pinnalla olevaa CA19-9:ä mittaavien määrityksien toiminnasta on lupaavia tuloksia, mutta ongelmana on, että käytetyt potilasnäytteet sisältävät vain pieniä määriä tällaisia yhdistelmiä. Määrityksien toimintaa ja erottelukykyä on vielä tarkoitus testata useammalla potilasnäytteellä.

Avainsanat: munasarjasyöpä, syöpädiagnostiikka, CA19-9, lateraalivirtausmääritys, vieritestaus

Hyvinvointia rakentamassa

Herkkä, leimakoetinkompleksin siirtoon perustuva määritys oligonukleotidianalyyttien havaitsemiseen

Maiju Liimatainen
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Mikro-RNA:t ovat 21–23 nukleotidin mittaisia, ei-koodaavia RNA-molekyylejä. Mikro-RNA:t ovat lupaavia diagnostisia merkkiaineita, sillä mikro-RNA:iden muuttuneiden pitoisuuksien on osoitettu olevan yhteydessä sairauksiin, esimerkiksi syöpiin, jo sairauksien varhaisessa vaiheessa. Mikro-RNA:iden alhainen pitoisuus veressä sekä pieni koko vaikeuttavat kuitenkin määritysten kehittämistä. Lisäksi pieniä pitoisuuksia määrittäessä käytetyn leiman epäspesifisestä sitoutumisesta aiheutuva taustasignaalin voi rajoittaa määrityksen herkkyyttä merkittävästi. Näiden ongelmien ratkaiseminen mahdollistaisi herkkien määritysten kehittämisen ja mikro-RNA:iden hyödyntämisen diagnostiikassa.

Työssä kehitettiin oligonukleotidianalyyttien havaitsemiseen määritys, jossa epäspesifinen tausta poistetaan kompleksin siirrolla. Työssä käytetty analyytti oli 34 nukleotidin mittainen DNA-oligonukleotidi. Määrityksessä koetinoligonukleotidilla pinnoitettu käänteisviritteinen nanopartikkeli sitoutuu analyytin toiseen päähän ja yhdessä nämä muodostavat leimakoetinkompleksin. Kiintokantajapinnalle kiinnitetty sitojakoetin sitoutuu analyytin vapaaseen päähän, jolloin leimakoetinkompleksi kiinnittyy sitojapinnalle. Pesun jälkeen leimakoetinkompleksi irrotetaan spesifisesti lisäämällä reaktioon sitojalle komplementaarista oligonukleotidia, joka syrjäyttää kompleksin sitojassa. Leimakoetinkompleksi vapautuu liuokseen, ja epäspesifisesti sitoutuneet nanopartikkelit jäävät sitojapinnalle. Vapautunut leimakoetinkompleksi siirretään ja sidotaan uudelleen toiselle sitojapinnalle, jolloin analyytille spesifinen signaali voidaan mitata ilman epäspesifistä taustaa.

Kompleksin siirrolla epäspesifinen taustasignaali saatiin poistettua lähes kokonaan, mikä paransi merkittävästi määrityksen herkkyyttä. Kompleksin siirtomäärityksellä analyyttinen herkkyys oli 73 amol/l ja parannus vertailumääritykseen, jossa leimakoetinkompleksia ei siirretä, oli viisisataakertainen. Proteiinipitoisessa näytematriisissa määrityksen herkkyys oli 800 amol/l, ja parannus vertailumääritykseen oli sataviisikymmentäkertainen. Tulokset osoittavat leimakoetinkompleksin siirron onnistuvan ja kehitetyn määrityksen toimivaksi. Käytetty DNA-analyytti on kuitenkin vielä tyypillistä mikro-RNA:ta pidempi, joten sitojakoettimia ja kompleksin syrjäyttävää oligonukleotidia on vielä kehitettävä lyhyempiä analyyttejä varten.

Asiasanat: epäspesifinen sitoutuminen, hybridisaatiomääritys, jalansijavälitteiset korvausreaktiot, kompleksien siirto, oligonukleotidit

Tunnistussilmukan kiinnittymisen ja sekvensointikirjastojen optimointi syövän diagnostiikassa

Rita Ojaniemi
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Syöpään sairastui maailmanlaajuisesti 19,2 miljoonaa ja kuoli 10 miljoonaa ihmistä vuonna 2020 IARC:n arvioiden mukaan. Syövän aikainen diagnoosi parantaa huomattavasti syövästä parantumisen mahdollisuuksia.
Uuden sukupolven sekvensoinnilla (engl. Next generation sequencing, NGS) on ollut suuri merkitys syövän diagnostiikassa jo nyt. Menetelmän herkkyys, nopeus ja alhaiset näytekohtaiset kustannukset tekevät menetelmästä kiinnostavan muihin diagnostiikkamenetelmiin verrattuna.
Diplomityössä optimoitiin tunnistussilmukan (indexing-Loop) kiinnittymistä silta-koetinkompleksiin. Tunnistussilmukka-silta-koetinkompleksilla pyydystetään genomialueet, joissa on kliinisesti merkittäviä mutaatioita, jonka jälkeen muodostunut kompleksi voidaan monistaa pyörivän ympyrän monistuksen (engl. rolling circle amplification, RCA) ja PCRn avulla. RCA tekee ns. lineaarisen monistuksen, joka helpottaa kirjastojen luomista. Monistuksen jälkeen sekvensointidatasta haetaan tiettyjä kliinisesti merkittäviä mutaatioita ja joissain tapauksissa kvantitaatiolla on merkitystä.
Työssä kehitettiin tunnistussilmukka, jonka lopullisena tarkoituksena on saada käytössä olevista kaupallisista sekvensointimenetelmistä skaalattavampia ja edullisempia. Tämä on mahdollista, koska tunnistussilmukan avulla potilasnäytteet voidaan merkitä tunnetuilla sekvenssijuosteilla ja yhdistää eri näytteet yhteen jo menetelmäprotokollan varhaisessa vaiheessa. Prosessi yksinkertaistuu merkittävästi, jolloin myös reagensseja kuluu huomattavasti vähemmän. Siten kaupalliseen sekvensointiin mahtuu enemmän potilasnäytteitä kuin ennen ja samassa ajassa voidaan saada yhä useampi diagnoosi. Menetelmä sopii erityisesti syöpien aikaiseen diagnosointiin sekä seurantaan.
Tunnistussilmukka saatiin kiinnittymään siltakoetinkompleksiin ja sekvensointikirjaston tuottamasta datasta pystyttiin todistamaan kirjastojen puhtaus. Kiinnittymisen optimoinnissa kokeiltiin mm. erilaisia entsyymejä ja eri määriä syklejä PCRssä. Tunnistussilmukallisten silta-koetinkompleksien määrää saatiin kasvatettua, mutta tavoitteena on vielä suurempi prosentti kiinnittyneitä tunnistussilmukoita. Onnistunut tunnistussilmukan kiinnittyminen takaa paremman suoriutumisen skaalattavuudessa, pienemmät kustannukset näytettä kohden, ja mahdollisuuden käsitellä suuremman määrän näytteitä samassa sekvensoinnissa.

Avainsanat: cfDNA, NGS, nukleiinihappo, PCR, RCA

Funktionaalinen kasvainorganoidien viljelymenetelmä immuno-onkologisten lääkevasteiden tutkimuksessa

Eetu Välimäki
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Immuno-onkologisilla hoidoilla on saavutettu pitkäkestoisia kliinisiä vasteita monissa syövissä ja yksittäisillä potilailla, mutta niiden tehokkuus on edelleen rajallinen. Immuno-onkologisten syöpähoitojen tehokkuuden edistämiseksi ja hoitojen kohdentamisen tehostamiseksi tarvitaan syvempää ymmärrystä lääkeaineiden ja immuunijärjestelmän eri solujen suhteista yksittäisen potilaan tasolla. Viime aikoina on kehitetty lukuisia erilaisia kolmiulotteisia (3D) soluviljelymalleja, joilla mahdollistetaan syöpäsolujen viljely todenmukaisemmissa *in vivo* -kudosrakennetta jäljittelevissä olosuhteissa. Näissä 3D viljelymalleissa syöpäsolut polarisoituvat ja muodostavat sferoideja tai organoideja, jotka yhdessä erilaisten mikrokuviointiteknologioiden, kuva-analyysi- ja koneoppimistyökalujen avulla mahdollistavat korkean suorituskyvyn lääkeaineseulontaa sekä uusia työkaluja solubiologisten ilmiöiden analysoimiseen ja luokittelemiseen. Immuno-onkologisten lääkeaineiden tutkimuksen yhteydessä näitä menetelmiä voidaan mahdollisesti käyttää myös tutkittaessa syöpä - immuunisolu vuorovaikutuksia, immunologisten syöpähoitojen tehoa sekä adaptiivisen immuunivasteen aktiivisuutta.
Työssä kehitettiin ja sovellettiin korkean suorituskyvyn 3D-soluviljelymenetelmää, jonka avulla pystyttiin onnistuneesti tutkimaan funktionaalisesti potilasperäisten syöpäsolujen muodostamien organoidien lääkeherkkyyksiä sekä syöpä - immuunisolu vuorovaikutuksia eri lääkekäsittelyjen aikana *ex vivo*. Tämän lisäksi työssä luotiin viljelyalustalla muodostuneiden organoidien fenotyyppien luokittelemiseen koneoppimistyökalu, mikä mahdollisti organoidien morfologiaan sekä immuunisolujen aktiivisuuteen perustuvan luokittelun. Työn aikana kerätty kuvadata mahdollisti fenotyyppien luokittelemisen kahteen luokkaan, mutta kuvadatan kasvaessa työkalua voidaan soveltaa useampien fenotyyppien luokitteluun. Syöpäsolujen soveltuvuus organoidien muodostamiseen sekä toistettavien organoidien luominen havaittiin olevan merkittävässä roolissa lääkeherkkyysmäärityksen toimivuuden kannalta.

Avainsanat: 3D-soluviljely, immunoterapia, koneoppiminen, lääkeherkkyys-seulonta, mikrokuviointi, organoidit

DNA:n eristykseen ja puhdistukseen käytettävän kasetin valmistus 3D-tulostuksella infektiodiagnostiseen vieritestausjärjestelmään

Helea Junes
Ohjaajat: TkT. Satu Lahtinen ja Prof. Tero Soukka
BIOTEKNIIKKA DI

Nukleiinihapon osoitustestit (engl. nucleic acid amplification, NAA) ovat infektiodiagnostiikassa hyödynnettäviä spesifisiä ja herkkiä molekyylidiagnostisia (MDx) testejä, jotka edellyttävät monivaiheista ja vaativaa näytteen esikäsittelyä. Infektiotautien nopeaan diagnosointiin vieritestauksessa (engl. point-of-care, POC) on kehitetty kaupallisia MDx-POC-alustoja, joissa testikasettiin on integroitu näytteen esikäsittely ja NAA-testi, mutta niiden kehitystyötä hidastaa kasetin valmistus perinteisin menetelmin. 3D-tulostus tarjoaa nopean väylän testikasettien kehittämiselle ja valmistukselle, mutta vähäinen tieto tulostusmateriaalien ja -tekniikoiden soveltumisesta nestekäsittelyyn ja yhteensopivuudesta biokemiallisiin reaktioihin vaikeuttaa siirtymää lisäävään valmistukseen.

Työn tavoitteena oli toteuttaa 3D-tulostuksen keinoin toiminnallinen ja automatisoitu kasetti DNA:n eristykseen ja puhdistukseen. Työssä suunniteltiin ja valmistettiin ruuviekstruusio- ja stereolitografiatulostuksella nestekäsittelyyn soveltuva kasetti, johon integroitiin silikapinnoitteisiin superparamagneettisiin helmiin perustuva nukleiinihappojen eristys ja puhdistus, ja verrattiin kasetin suorituskykyä manuaaliseen kaupalliseen eristyssarjaan.

Työssä kehitetty suljettu kasettikonsepti (83 mm x 35,5 mm x 17 mm) hyödyntää ilmanpaineista nesteensiirtoa ja valitsijaventtiiliä sekä mahdollistaa reagenssien vapaan yhdistämisen, sekoittamisen ja lämmityksen erillisessä reaktiokammiossa. Erikseen 3D-tulostetuista osista koottu kasetti on nestetiivis myös pintajännitettä poistavilla nesteillä. Kasetin ohjaamiseen rakennettu analysaattori mahdollistaa tarkkojen tilavuuksien siirron ja kasetin toimintojen automatisoinnin. Alustavien tulosten perusteella kasetilla toteutettu eristys on vertailukelpoinen manuaaliseen eristykseen. Tulevaisuudessa tavoitteena on integroida kasettiin NAA-testi ja toteuttaa siten kokonainen MDx-POC-alusta 3D-tulostamalla.

Asiasanat: lisäävä valmistus, ruuviekstruusiotulostus, stereolitografia, näytteen esikäsittely, nukleiinihapon osoitustestit

Highly sensitive assay for long forms of cardiac troponin T to improve myocardial infarction diagnostics

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Patients with myocardial infarction (MI) require prompt care, but diagnostic challenges can delay the start of treatment. A commonly used biomarker for MI, cardiac troponin T (cTnT), is released in long forms during a MI from the damaged cardiac muscle to the blood circulation. Current cTnT tests measure various forms of cTnT and detect elevated cTnT concentrations in several patient groups. An assay specifically detecting long cTnT forms is better at discriminating MI patients from other patient groups. However, the available long cTnT assay is not sensitive enough. The aim of this thesis project was to develop a highly sensitive assay for long cTnT forms.

Based on two immunoassays previously developed at the University of Turku, a novel immunoassay with a photon upconverting label was developed to detect long forms of cTnT in plasma samples. Preliminary analytical performance characteristics of the assay were determined. Plasma samples from non-ST-elevated MI (NSTEMI, N=32), end-stage renal disease (ESRD, N=38) and atrial fibrillation (AF, N=41) patients were analyzed to evaluate the ability of the assay to distinguish these patient groups.

High assay sensitivity was achieved, based on the preliminary limit of blank (0.15 ng/l), limit of detection (0.39 ng/l) and limit of quantitation (1.79 ng/l, 10 % CV). Unlike the standard cTnT test currently used in hospital laboratories, the developed assay was able to distinguish NSTEMI patients from both ESRD ($p<0.001$) and AF patients ($p<0.001$). This study demonstrates that the specific detection of long cTnT forms could improve MI diagnostics.

Keywords: cardiac troponin T, myocardial infarction, upconverting nanoparticles

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Nanoparticle aided glycovariant biomarkers for monitoring lung cancer

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Lung cancer is no. 1 in cancer related cause of deaths worldwide in both sexes, according to WHO. Lung cancers high mortality rate is mainly caused by late diagnosis. Most patients have stage III or IV disease at the time of diagnosis. Most common treatments for lung cancer are surgery, radiofrequency ablation, radiation therapy, chemotherapy, targeted drug therapy, immunotherapy, and palliative procedures. The ability to monitor cancer response to various treatments would help in selecting the most effective therapy forms.

Glycoproteins are cells main components. Glycosylation enables high variation of different protein functions. Numerous glycoproteins are used as cancer biomarker with different cancers. However, because they are also found in other benign disease conditions along with healthy individuals and hence lack specificity. Cancer cells differ highly from normal cells, they also have their own altered glycosylation patterns. These altered glycoproteins are specific to the cancer and are highly promising target to search for new biomarkers.

Glycan binding proteins include for e.g., lectins and antibodies that bind specifically to certain glycan structures. They are found in all living organisms and hence have highly variable targets. In this study, we use lectins to find different glycoforms of CA19-9, CA15-3, and CA125 glycoproteins from lung cancer patients EDTA plasma samples. Due to the low dissociation constant of lectins, we coat them on highly fluorescent europium chelate dyed nanoparticles to increase the binding affinity. Seven different capture $F(ab)_2$ were used to track different glycoproteins: C192, C241, C242, Ma552, Ma695, Ov185 and Ov197 and six different lectins to detect different glycoforms: MGL, MBL, TJA II, AOL, WGA, and UEA I.

We found that three CA19-9 glycovariants (C192-WGA, C241-TJA II and C241-AOL) and one CA15-3 glycovariant (Ma552-TJA II) followed most effectively the progressive and regressive lung cancer disease in patients over time. Standards were established and the glycovariant assays showed good linearity. Results were compared to CA19-9 and CA15-3 commercial kits from Fujirebio and it was observed that it is beneficial to detect specific glycovariant instead of the glycoprotein itself. Overall, these four glycovariant based assays show potential for use in monitoring of lung cancer.

Keywords: lung cancer, biomarker, glycovariant, lectin, immunoassay, nanoparticles

Detecting cardiac troponin T forms in advanced chronic kidney disease patients

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Cardiac troponin T (cTnT) assays are widely used in the diagnostics of myocardial infarction. Chronic kidney disease (CKD) is a progressive disease in which the filtration rate of kidneys is impaired. CKD patients have stable elevations of cTnT in their bloodstream. Circulating cTnT is in fragmented form in end-stage renal disease while in myocardial infarction cTnT is found in long molecular forms. CTnT forms in advanced CKD have not been studied. The aim of this study was to develop an immunoassay for total cTnT measurement and to determine the amount of total cTnT and long cTnT in advanced CKD patient samples.

In the development of the total cTnT assay different biotinylated and upconverting nanoparticle (UCNP) conjugated antibody combinations were evaluated in a heterogeneous sandwich immunoassay. Heparin plasma samples from cardiac patients with known commercial total cTnT test results were used for the assay development. The advanced CKD patient heparin plasma sample panel (n=152) was analyzed with the optimized total cTnT assay and with an assay detecting only long forms of cTnT.

The antibodies which gave the best concentration correlation to a commercial total cTnT assay were chosen (Spearman's r 0.90) for the total cTnT assay. Analytical detection limit of 0.51 ng/L (zero calibrator+3SD) was reached with the optimized total cTnT assay. The CKD patient sample panel results for the total cTnT assay were (median [25^{th}–75^{th} percentile]) 38.7 [23.2–67.3] ng/L and for the long cTnT assay 1.9 [1.2–3.0] ng/L. The fraction of long cTnT in total cTnT was 4.3 [3.3–6.7] %. The results confirmed that advanced CKD patients have elevated total cTnT concentrations, but this was the first study to reveal that this elevation is predominantly caused by short cTnT fragments. The study highlights that CKD patients cannot be diagnosed with myocardial infarction by using a total cTnT test but long cTnT test could be used instead.

Keywords: cardiac troponin T, chronic kidney disease, UCNP

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