Joint Glycobiology Meeting
18-20 September 2022

Program

https://nvbmb.kncv.nl/glyco32

#JGMglyco32

CONTENT

	Page
Practical information	**3**
Venue	3
Travel directions by public transport	3
How to get there by car	3
Group transport and taxi services	4
Sightseeing in Utrecht	4
Checking in at the conference	5
For presenters	**5**
Oral presentations	5
Poster presentations	5
Social program	**5**
COVID-19 safety measures	**6**
Program at a glance	**7**
From our sponsors	**9**
Oral presentations with abstracts	**17**
Posters with abstracts	**40**
Poster list	**73**
List of attendees	**74**

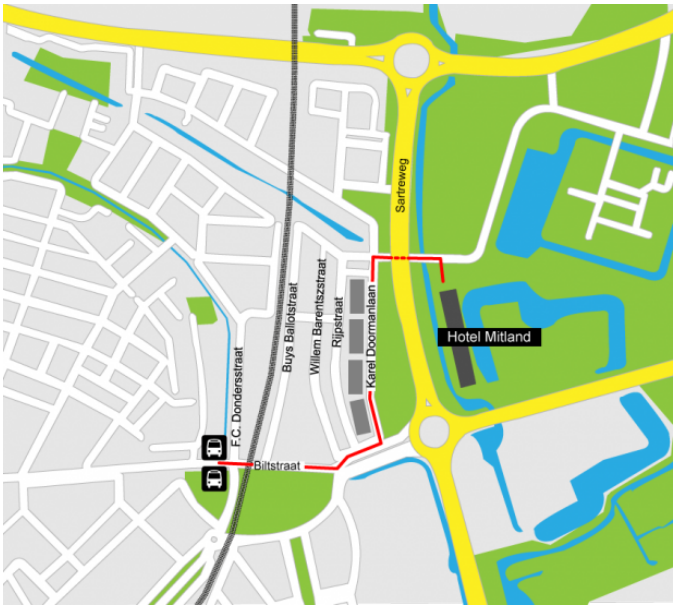
Practical information

Venue
Hotel Mitland
Ariënslaan 1
3573 PT Utrecht
Phone: +31 (0)30 271 58 24
Email: info@mitland.nl

Travel directions by public transport

From Amsterdam Schiphol Airport
- Trains depart for Utrecht Central Station every 15 minutes

From Utrecht Central Station
- Take bus 50, 73, 74 or 77, these buses run average every 10 minutes
- Get off at the Oorsprongpark stop, from this stop it is about 10 minute walk
- Cross the railway tracks
- Turn into the fourth street on the left, this is the Karel Doormanlaan, and walk straight along the apartment buildings
- After approximately 350 meters, turn right at the crossroads and continue under the Voorveldse Polder viaduct to the Ariënslaan
- You will find Hotel Mitland after approximately 50 meters on the right



For information on public transport in The Netherlands: http://9292.nl/en

How to get there by car

From Breda
Take the A27 in the direction of "Ring Utrecht (Oost) / Hilversum / Almere". Follow the 'Veemarkt' signs at Utrecht. Take the 'Veemarkt' turning (exit 30). At the traffic lights at the T-junction, turn left and go under the viaduct. You will pass a 'Firezone' petrol station on your right. Drive all the way round the first roundabout, you come to so that you are driving back in the same direction as you came. Immediately after the roundabout (about 70 meters) take the first right turn. Turn right again at the T-junction. This is the Ariënslaan. After about 300 meters, you will see Mitland Hotel Utrecht to your left.

From 's-Hertogenbosch
At the 'Everdingen' crossroads (where the A27 and the A2 split up) a good 10 km before Utrecht, follow the Utrecht / Almere (A27) signs. Stay on the A27 until you see the 'Veemarkt' signs when level with Utrecht. For further directions, see directions 'From Breda'.

From Amsterdam
Follow the A2 to the 'Oudenrijn' intersection. Then follow the 'Ring Utrecht' signs which will bring you on to the A12. Follow the 'Ring Utrecht' signs here too. At the 'Lunetten' intersection, follow the 'Ring Utrecht / Amersfoort / Hilversum (A27)' signs. For further directions, see 'From Breda'.

From The Hague
Follow the A12 as far as the 'Lunetten' intersection. Take the 'Ring Utrecht / Amersfoort / Hilversum (A27)' turning. Once on the A27, follow the 'Veemarkt' signs. For further directions, see 'From Breda'.

From Hilversum
Take the A27 in the direction of Utrecht. Follow the 'Veemarkt', Centrum, de Bilt" signs at exit 30 when level with Utrecht. You will pass a 'Firezone' petrol station on your right. Drive all the way round the first roundabout you reach so that you are driving back in the same direction as you came. Take the first right turn immediately after the roundabout (about 70 meters). Turn right again at the T-junction. This is the Ariënslaan. After approximately 300 meters, you will see Mitland Hotel Utrecht to your left.

From Amersfoort
Take the A28 in the direction of Utrecht. At Utrecht, follow the "Ring Utrecht (Noord) / Veemarkt / Hilversum (A27)". Take the 'Veemarkt' turning (exit 30). For further directions, see 'From Breda'.

From Arnhem
Take the A12 in the direction of "Ring Utrecht (Oost) / Hilversum (A27)". On the A27, follow the 'Veemarkt' signs. For further directions, see 'From Breda'.

Group transport and taxi services

On request we can organize paid group transportation for excursions. Also, group transportation to and from Hotel Mitland is possible. When ordering a taxi in Utrecht we recommend UTC (Utrechtse Taxi Centrale). Are you travelling from or to Schiphol Airport? Please consult our reception for more information to prevent unnecessary high costs.

Sightseeing in Utrecht

The Domtower
The Domtower is the highlight of Utrecht city center. The building of this magnificent gotic church tower was initiated in 1321. The Domtoren is currently being restored, but can still be visited. Book a visit at https://www.domtoren.nl

Domsquare
The Domsquare is the central square of Utrecht where the Domtower is situated.
Next to the square, you can also find the Academy building were all official ceremonies of Utrecht University take place. You can walk into the garden of the Academy Building.

Oudegracht
The Oudegracht is the central canal in Utrecht city center. You can walk along the canal with its old storage docks (called “werfkelders”) or explore the canal by pedal boat or boat tour. Many restaurants are situated in the storage docks that can be reached by stairs.

Pedal boats:	https://www.stromma.com/en-nl/utrecht
Boat tours:	https://www.schuttevaer.com

Examples of restaurants at the docks:

Vegetarian Restaurant de Werfkring	https://www.dewerfkring.com/#restaurant
German restaurant Kartoffel	https://kartoffel.nl
Broadway Steakhouse	https://www.steaksandribs.nl

Museums in Utrecht

Railway Museum Utrecht https://www.spoorwegmuseum.nl/en/
A museum for train enthusiasts.

Museum Speelklok https://www.museumspeelklok.nl/lang/en/
An interactive museum with typical Dutch self-playing musical instruments.

Checking in at the Conference

Early check-in: Sunday 18th September, 16.00-20.00 pm at Hotel Mitland

Late check-in: Monday 19th September, 07.30-8.30 am at Hotel Mitland

For presenters

Oral presentations
Please see session chair 30 minutes prior to the start of your session.
Bring your presentation on a memory stick.

Posters
Dimensions: Portrait format, maximum 90 cm x 110 cm (approx. 35" x 43")
Poster should be put up in poster room on Sunday 18th September, 15.00-20.00
Poster should be removed by Tuesday 20th September, 14.30.

Social program

Sunday 18th September
19.00 - Welcome drinks
Location: Hotel Mitland
Having a warm welcome for our friends that needed to travel from Lille, Germany...and other far-away places.

Monday 19th September
19.00-23.00 Dinner followed by Bowling
Location: Bowling lanes at Hotel Mitland
Join in for a friendly bowling competition with your fellow glycobiologists!. The bowling lanes are on-site at hotel Mitland. Please sign up for bowling at the registration desk.

COVID-19 safety measures (last updated 2022-06-23)

NOTE! Please visit https://www.government.nl/topics/coronavirus-covid-19 for the latest rules and regulation for entering the Netherlands.

Travel to Netherlands:
There are no coronavirus-related restrictions for entering the Netherlands for travelers who live in the EU/Schengen area or in a country participating in the EU travel rules scheme. The EU entry ban applies to other travelers who live outside the EU/Schengen area, but there are some exceptions.

For travelers from outside EU/Schengen area, e.g. Israel, Japan, UK, USA:

1. You may only enter the Netherlands if you're fully vaccinated.
2. Since the 23rd of March you no longer need to show proof of test when you travel (back) to the Netherlands.
3. Since the 19th of April you no longer have to show a health declaration when boarding a plane or ferry.

A corona test can be booked at www.spoedtest.nl.

Available test locations include St. Jabobstraat 247 in Utrecht and Hoeksteen 149, location Schiphol Airport/Hoofddorp. Different test options are available with results within 1 hour or 1 day and also include travel documents.

COVID policy at Mucins in Health and Disease Conference

Face masks
There is currently NO face mask policy in the Netherlands, therefore wearing a face mask is optional. The conference will supply a limited number of FFP2 and N95 masks for participants.

Symptoms and testing
In case of symptoms, perform a self-test. The conference will supply a limited number of kits, but attendees are encouraged to bring test kits for personal use. A corona test can also be booked at www.spoedtest.nl

If symptomatic and the test is negative, wearing a face mask and practicing physical distancing measures are encouraged.

If the test is positive, quarantine yourself and follow the RIVM instructions. You must quarantine and unfortunately cannot participate in the program anymore. Quarantine can be lifted after 24 hours without symptoms OR 10 days after the start of the symptoms.

For more information on the Dutch official policy, visit:
https://www.government.nl/topics/coronavirus-covid-19
https://www.rivm.nl/en/coronavirus-covid-19/current-information

Program

Monday 19th September
Glycoimmunology (9:00 – 11:00)

0900 – 0940 Keynote lecture **Yvette van Kooyk** (Vrije Universiteit, Amsterdam)
Glycocode of the tumor microenvironment a novel immune checkpoint

0940 - 0955 **Oren Moscovitz** (Max Planck Institute of Colloids and Interfaces) *Generation of glycan-specific nanobodies*
0955 - 1010 **Ronald Derking** (University of Amsterdam)
Glycans as a tool to reduce off-target epitopes on HIV-1 Env trimers
1010 - 1025 **Angelina Kasprowicz** (University of Lille)
Deciphering and targeting GD2 ganglioside O-acetylation pathways in neuroectoderm-derived cancers
1025 – 1055 **Vered Padler-Karavani** (Tel Aviv University, Israel)
Immunological responses against glycosylated biotherapeutics and biodevices in humans

Coffee break (11:00 - 11:30)

Glycans in host-pathogen interactions (11:30 – 13:00)

1130 – 1210 Keynote Lecture **Marthe Walvoort** (Groningen University)
Extracellular Bacterial Glycans: the Good and the Bad

1210 - 1225 **Mariana Juárez Osorio** (Universitat Magdenburg)
Towards full control over protein glycosylation: in-vitro glycoengineering of influenza a virus hemagglutinin and sars-cov-2 spike protein
1225 - 1240 **Astrid Hendriks** (University of Amsterdam)
Mapping the antibody repertoire to Staphylococcus aureus wall teichoic acid reveals a protective role for IgM during invasive infection
1240 - 1255 **Tatiana Shamorkina** (Utrecht University)
Glycan shielding on Dengue and Zika virus envelopes against broadly neutralizing E-dimer epitope (EDE) antibodies revealed by mass spectrometry

Lunch & poster session (13:00 - 15:00)

Glycotechnology and analytics (15:00 – 17:00)
1500 – 1540 Keynote Lecture **Max Crispin** (University of Southampton)
Viral glycosylation: from HIV to SARS-CoV2

1540 - 1555 **Noortje De Haan** (University of Copenhagen)
The use of mass spectrometry and glycogenomics for the dissection of the human O-glycome
1555 – 1610 **Ward Doelman** (Leiden University)
Glyco-PAINT: quantifying glycan-lectin binding kinetics at the single molecule level on living cells
1610 - 1625 **Tuan Huang Son** (University of Magdenburg)
In-vitro multi-enzyme cascades for the synthesis of uridine nucleotides sugars

1625 - 1640 **Daniel Hornikx** (Radboud University)
Light-controlled spatiotemporal desialylation with a photoactivatable sialyltransferase inhibitor

Bussiness meeting – break – poster & refreshments

Dinner and bowling 19:00 – 22:00

Tuesday 20th September

Mucosal glycans and O-linked glycosylation (9:00 – 10:30)

0900- 0940 Keynote Lecture **Christian Bull** (Radboud University)
Mucin O-glycosylation cell-based display

0940 – 0955 **Marco Albers** (Hannover Medical School)
Why are Cys domains of mucins not C-mannosylated
0955 - 1010 **Daphne Stapels** (Utrecht University)
MUC13: a fine, sweet, line between epithelial maintenance and tumorigenesis
1010 – 1025 **Julia Beimdiek** (Hannover, Germany)
Role of glycosphingolipids in development

Coffee break/poster session (10:30 – 11:30)

Congenital disorders of glycosylation (11:30 – 13:00)

1130 – 1210 Keynote Lecture **Hans Wessels** (Radboud University)
Plasma glycoproteomics for patient diagnostics and delineation of disease glycobiology

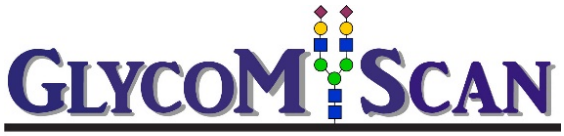
1210 - 1225 **Ulla Gerling Driessen** (University of Dusseldorf)
Multi-TaG: A modular platform of bioorthogonal probes for multi-modal glycoprotein analysis
1225 - 1240 **Charlotte Althoff** (University of Lille)
Deficiency of Pseudoautosomal DHRSX Sheds a New Light on Dolichol Metabolis
1240 - 1255 **Merel Post** (Radboud University)
Deciphering sialic acid pathway regulation via in-depth multi-omics approach in tissue-specific human models

Poster prizes - announcement - Lunch - Goodbye

We are grateful to our Dutch Organization for Scientific Research (NWO) for their support for this meeting and the glycobiology community

From our sponsors

Services and contract research in analytical mass spectrometry for pharma and life sciences



Monique van Scherpenzeel, PhD
CEO and founder

GlycoMScan was founded in 2018 and its service laboratories are located at the Pivot Park in Oss. We offer method development and highly sensitive and quantitative analyses in several types of biological samples like plasma, tissues, urine, cell culture media and cell extracts. We perform pharmacokinetics including pharmacokinetic modelling, and provide high level of scientific knowledge and advice. Protein glycosylation is our field of expertise.

With more than 15 years of experience in science we offer high quality contract research.

Customers like our **short communication lines** and high-quality research. We listen to the customer, discuss and develop tailor-made solutions according to their needs. We are a **genuine partner** in the research of the customer, and thus offer our honest scientific advice and experience for the customers' benefit. We are proud that our network is growing by "word of mouth".

Services and Products

- What services do we offer?
    - Protein glycosylation and glycolipid analyses
    - Mono- to oligosaccharide including RNA, nucleotide sugars and glycan analyses
    - Pharmacokinetics
    - Quantification of drugs and metabolites in plasma, tissues, cells, hair, urine etc.

- What products do we make?
    - Analytical method development and LC-MS analyses

Our Expertise

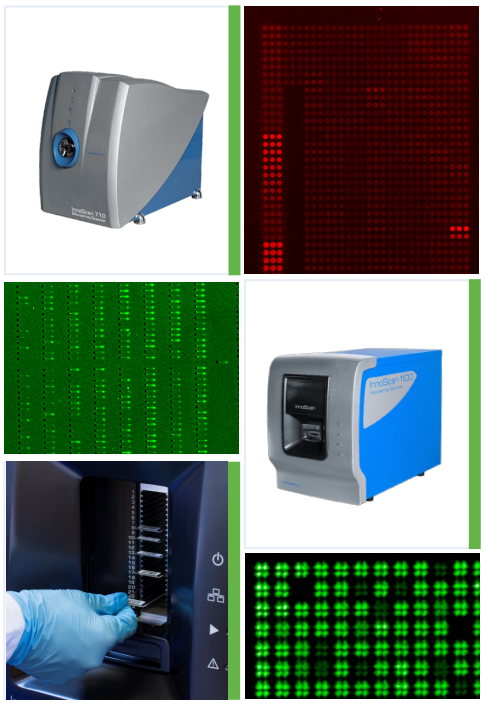
- Mass spectrometry
- Chromatography
- Protein glycosylation
- Omics: proteomics, metabolomics and glycomics
- Analytical chemistry
- Biochemistry
- Clinical Chemistry

INNOSCAN SCANNERS

A complete range of fluorescence scanners

Innoscan key features

- High performance fluorescence scanners
- Open to all kinds of microarray applications
- Easy multiplexed assays
- User-friendly software
- Single slide and 24-slide autoloader systems
- Complete range of scanners to adapt to each microarray slide format and application



Adapted focusing to different applications and sample formats

Made in France

Certification

Public and Privates Centers

+ 50 contries

INNOPSYS - Parc d'Activités Activestre 31390 Carbonne France - Phone: +33 561 971 974 - contact@innopsys.fr
INNOPSYS INC - 965 W Chicago Ave. IL 60642 Chicago USA - Phone + 1 312 806 8535 - contact@innopsys.com

Funded by Seedfinancing from BMDW/BMK through the Austrian Wirtschafts Service.
Bundesministerium Klimaschutz, Umwelt, Energie, Mobilität, Innovation und Technologie
Bundesministerium Digitalisierung und Wirtschaftsstandort
austria wirtschafts service aws

CUTANOS

Introducing the LC-TDS: A Cell-specific Antigen Delivery Platform for Immune Modulation

Who we are

CUTANOS GmbH is a biotech company founded by Robert Wawrzinek and Christoph Rademacher in early 2021 focussing on the development of antigen-specific vaccines and new treatments for autoimmune disorders. Cutanos uses a modular and highly flexible drug delivery system called the Langerhans Cell Targeted Delivery System (LC-TDS). With this technology, the company aims to realize innovative immunotherapies in conjunction with minimally invasive administration. As a spin-off of the Max Planck Institute of Colloids and Interfaces, Cutanos will demonstrate the full range of its platform technology from their headquarter in Austrian capital of Vienna, financially backed by an investment consortium (including KHAN-I, HTGF and IST cube) and an AWS Seedfinancing.

Robert Wawrzinek
Co-Founder & Managing Director

Entrepreneur and scientist trained in organic chemistry with 10 years of experience in the fields of biomedicine, material science and spectroscopy. He worked at high-ranking international research institutions, published some 25 articles in peer-reviewed journals and filed two patents. Robert's core competences include strategic project design, risk assessment, project management, team coordination, communicating and networking, as well as practical lab work in chemistry and biochemistry.

Christoph Rademacher
Co-Founder & Advisor

Accomplished researcher with a strong background in biotechnology and the main inventor of the LC-TDS technology. He was an independent group leader at the Max Planck Institute of Colloids and Interfaces Potsdam and was recently appointed full professor for Molecular Drug Targeting at the University of Vienna and Max F. Perutz Labs. Christoph maintains an interdisciplinary network of academic collaborators and is well connected to industrial partners worldwide.

Ulrich Platte
CFO/CBO

Executive C-level manager with proven record of establishing cross functional partnerships to deliver results. Innovative, agile, global strategic leader driving operations within marketing sales and business development. Ensure customer focus and profound expertise in life science industry.

Federica Quattrone
Senior Scientist & Project Leader

Biotechnologist specialized in Molecular Medicine by training and further educated in European Patent Law. She carried out doctoral studies in Immunology at the Medical University of Vienna and at CeMM, where she developed a meaningful network across Viennese Start-ups and Academia. Federica further coordinates own-asset developments, external collaboration projects and represents Cutanos at international conferences.

Florian Sparber
Senior Scientist & Project Leader

Scientist with more than 12 years of experience in skin immunology, dendritic cell biology and infectious diseases. Worked at top research institutions and published numerous articles in international and renowned journals. Gained expertise in drug development, marketing and legislation for inflammatory skin disorders as a medical advisor in the pharmaceutical sector. Florian is responsible for research project development, *in vitro* and *in vivo* experimentation, animal model development, communication and networking.

Klara Klein
Scientist

Mirza Sarcevic

Gabriele Carta

Alexandra Sykora
Office Manager

Research Assistants

Technology

Challenge

Traditional therapeutics are administered by injection into blood or muscle and passively find their way to their target cells – after most of the drug has already been cleared or was taken up by numerous "off-target cells". This leads to a low clinical efficacy and requires administration of higher dosages followed by an increased risk of unwanted side effects. Targeted delivery of defined antigens to specialised immune cells promises to overcome these drawbacks. However, this is difficult to achieve since ligands to selectively target certain immune cells are sparse.

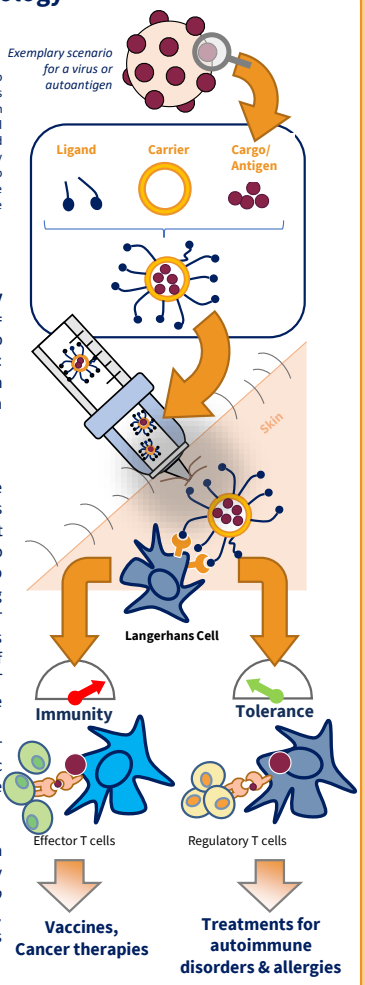
Solution

Cutanos' **Langerhans Cell Targeted Delivery System (LC-TDS)** can deliver various cargos (= antigens such as protein, peptides or mRNA) to a specific subset of skin-residing immune cells: Langerhans cells (LCs). The modular system further comprises of a vehicle decorated with the first LC-specific ligand.

Working Principle

By using FDA-approved and minimally invasive micro-needle application devices, the LC-TDS is painlessly administered right where the target cells reside – the upper most skin layer. Due to an ideal ligand affinity the cargo is released into the inner cell body of LCs for further processing and without blockage of the targeted receptor for additional uptake cycles (as antibodies would do). This accumulates large quantities of antigen, which are eventually presented to T cells in the lymph nodes, eliciting the immune response.
Moreover, as LCs induce tolerance in their steady-state, Cutanos explores the therapeutic potential of their platform for autoimmune disorders and allergies.

The LC-TDS' proof-of-concept has been extensively demonstrated in model and primary cell lines, human skin explants and first *in vivo* experiments. Further preclinical evaluations, including other animal species and indications are currently ongoing and very promising.



Opportunities

In a viral pandemic scenario, the LC-TDS reduces the required vaccine dose making better use of production-limited antigen and improves patient compliance by minimal invasive application paired with reduced side effects.
By controlling the activation state of Langerhans cells, the LC-TDS aims to regain tolerance against self-antigens in autoimmune disorders. This embodies a particularly medical need since there is currently no cure for any autoimmune disease.
Since this versatile delivery system can be used to generate potentially any antigen-specific immune response, it is compatible with next-gen mRNA therapeutic and prophylactic vaccines and cancer immune therapies, as well as protein- and liposome-based technologies.

Our Funding Partners

KHAN-I · IST cube · High-Tech Gründerfonds · + A Private Investor · austria wirtschafts service aws

Get in touch:
Cutanos GmbH | Josef-Holaubek-Platz 2 (UZA II), A-1090 Vienna | office@cutanos.com | +43 676 576 0186
www.cutanos.com · twitter.com/cutanos · linkedin.com/company/cutanos-gmbh

PROTEIN METRICS
by Dotmatics

The Byos software program provides a one touch platform to launch multiple workflows across protein and oligonucleotide characterisation, allowing scientists to move from raw file to report in minutes.

Byos excels at providing analysis, inspection, automation and reports - making it easy to not only characterise your molecules, but to be alerted of changes to critical attributes.

Byos provides workflows from intact, sub-unit and native analysis to peptide level analysis, to chromatogram analysis, to de novo sequencing analysis, amongst others.

Underlying many of these workflows is Byonic™. Byonic is the industry leading full MS/MS search engine providing unequalled sensitivity for comprehensive peptide, protein and glycopeptide identification.

Find out more about how Byonic performed in a recent cross laboratory study by:

HUMAN GLYCOPROTEOMICS INITIATIVE

SCAN ME

Effective nutrients to maximise performance of microbial and mammalian cells

Kerry is a leading manufacturer of supplements for microbial and mammalian cell culture media that ensure optimal cell growth, high yields and growth consistency.

Providing solutions for Cell Culture and Fermentation has been our focus for more than 75 years.

Our broad portfolio for microbial and mammalian culture media consists of:
- Protein Hydrolysates (from Vegetable, Ultra-filtered, Dairy and Animal origin)
- Yeast Extracts (non-Ultra-filtered and Ultrafiltered)
- Recombinant, animal component free Proteins
- Optimized solutions for specific application needs, such as CHO cells, Insect cells and *E.coli* cells

Our solutions for Mammalian cells:
- stimulate cell growth
- increase protein production
- replace or reduce the use of serum
- significantly shorten process development times

Our solutions for Microbial cells:
- provide optimized nutrients for a broad range of microorganisms
- replace the use of animal components
- stimulate cell growth
- increase productivity

Would you like to learn more? Contact us at: **kerry.com/pharma**

Oral presentations

Glycocode of the tumormicroenvironment

a novel immune check-point

Yvette van Kooyk PhD

Department of Molecular Cell Biology and Immunology, Amsterdam UMC, VU University medical Centre, Amsterdam UMC, de Boelelaan 1108, 1081HZ Amsterdam, the Netherlands

E-mail: y.vankooyk@amsterdamumc.nl

Alterations in glycosylation is a hallmark in pathogen recognition by the host which mediates cellular communication during inflammation but also the resolution-phase of inflammation. Homeostatic and inflammatory processes affect the glycan signature of glycoproteins expressed on the cell surface, or secretory proteins, due to up- and down-regulation of glycosylation genes. We study those glycan signatures that are recognized by glycan binding receptors such as C-type lectins and Siglecs expressed on diverse set of myeloid and lymphoid immune cells, that modify intracellular signalling and immunological outcome. In particular the microenvironmental glycosylation signatures (the glycocode) that alter during inflammation and cancer are studied that drive immune responses towards immunity or tolerance and open new venues for immune interference.

We identified new mechanisms of immune tolerance through the modification of glycosylation of tumours (melanoma, pancreatic cancer and glioblastoma). In particular high expression of sialylation of tumours results in the increase of FoxP3 CD4+ T cells (Treg), differentiation of monocytes into TAMs and lower frequencies of effector T cells (Teff) and NK cells at the tumour site. In contrast, low sialylation of tumours converts the frequencies Treg/Teff to favourable anti-tumour immunity. These sialic acids can be used for the active induction of antigen specific immune tolerance by DC through Siglecs when coupled to a specific antigens, such as OVA, or peptides of MOG or derp-1 thereby setting the resolution phase and repairing auto-immunity such as multiple sclerosis or allergies.

Post-translational processes such as glycosylation, uncover new communication between cancer and immune cells. Because these glycosylations can be immune stimulatory or inhibitory we implement our discoveries in the treatment of cancer and auto-immune diseases.

Immunological responses against glycosylated biotherapeutics and biodevices in humans

Vered Padler-Karavani[1]

[1]Departments of Cell Research & Immunology, The Shmunis School of Biomedicine and Cancer Research, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

Abstract

Susceptibility to structural valve deterioration is one of the major drawbacks of bioprosthetic heart valves (BHVs). *N*-glycolylneuraminic acid (Neu5Gc) is an immunogenic dietary-carbohydrate antigen in humans because of inactivation of the gene encoding CMP-*N*-acetylneuraminic acid hydroxylase (*CMAH*), and all humans have circulating anti-Neu5Gc antibodies. We hypothesized that interaction of anti-Neu5Gc antibodies with Neu5Gc on BHVs could lead to immune response resulting in valve deterioration through calcification. We demonstrate Neu5Gc in both native calcified human valves as well as in calcified-BHVs, explanted from human patients, by HPLC and immunohistochemistry. Furthermore, anti-Neu5Gc IgGs were purified from native calcified human valves, validated by a glycan microarray. In the Neu5Gc-free *Cmah*-KO mouse model, anti-Neu5Gc antibodies promoted calcium deposits in subcutaneous implanted BHV discs, both with passive transfer of affinity-purified human anti-Neu5Gc IgGs, and by active-immunization of *Cmah*-KO mice with Neu5Gc-containing glyconanoparticles. Thus, co-existence of Neu5Gc/anti-Neu5Gc likely mediate BHV structural valve deterioration.

molecules.

Extracellular Bacterial Glycans: the Good and the Bad

Prof. Marthe T.C. Walvoort

Stratingh Institute for Chemistry, University of Groningen, the Netherlands

Cells across all kingdoms of life are covered with a dense layer of glycans, called the 'glycocalyx'. This layer contains a large variety of glycan structures, including polysaccharides, proteoglycans, glycolipids and glycoproteins. The glycocalyx is highly variable among organisms, cell types and growth phase. As a result, the glycocalyx is a crucial component in the recognition of and communication between cells.

On our research, we aim to establish proof that specific glycan structures are responsible for bacterial function, and I selected two different glycan structures for this lecture. The first type is the glycoproteins produced by Gram-negative *H. influenzae* bacteria. These adhesin proteins are densely covered by glucose residues, which are important to establish bacterial adhesion to host cells. By reconstituting this system *in vitro,* we established that the glucose residues are attached in a semi-processive manner,[1] which is a highly unusual mechanism for enzymatic glycosyl transfer.[2]

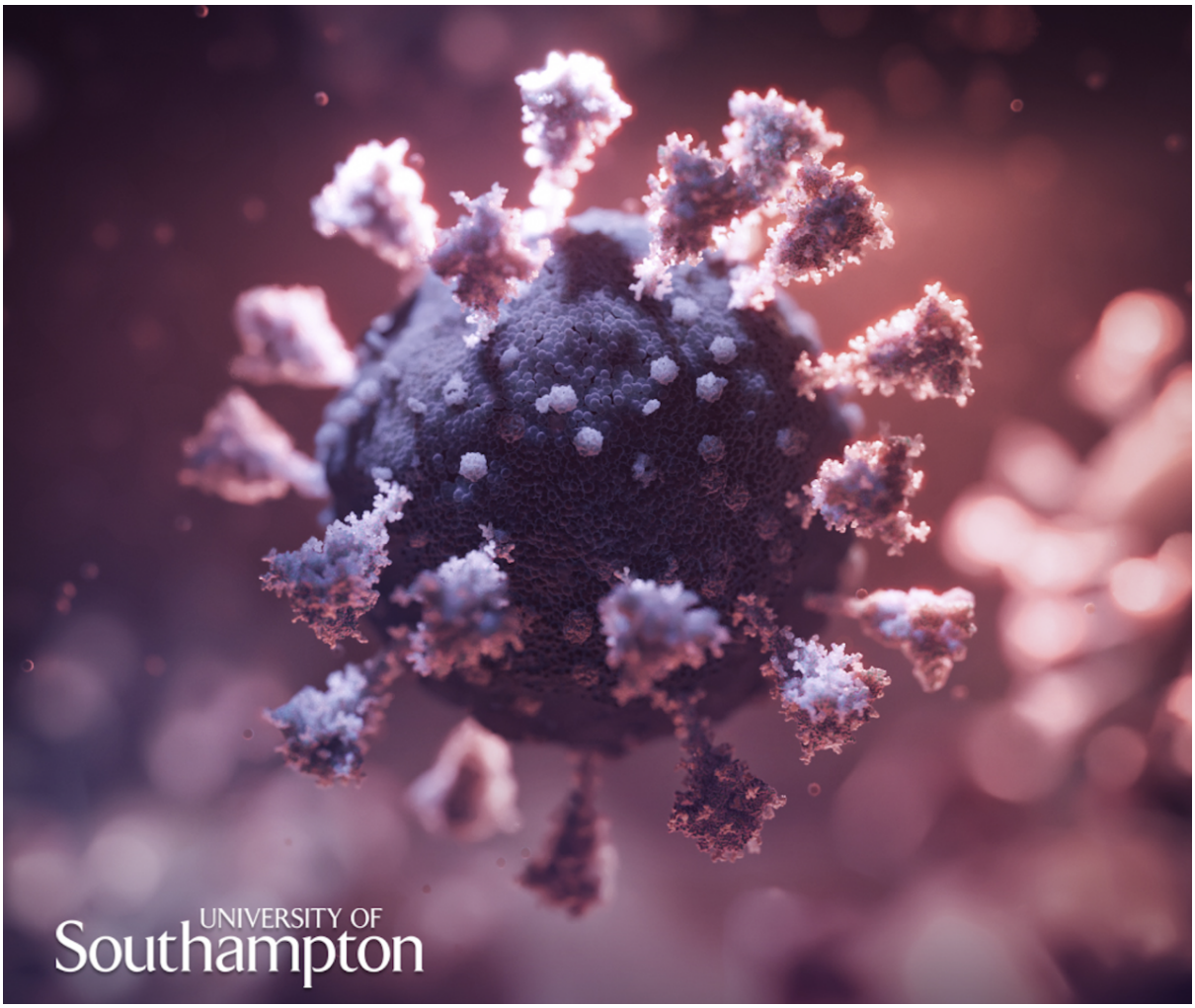
The second type of glycan structures is the exopolysaccharides from Gram-positive bifidobacteria. Evidence is emerging that exopolysaccharides from probiotics play an important role in establishing and maintaining gut health.[3] We developed a straightforward method to isolate exopolysaccharides from various commercially available bifidobacteria,[4] and are currently investigating their impact on intestinal epithelial cells, also in connection to pathogen adhesion.

[1] Yakovlieva, L.;Ramirez-Palacios, C.; Marrink, S.J.; Walvoort, M.T.C. *ACS Chemical Biology* **2021**, *16*, 165-175.
[2] Yakovlieva, L.; Walvoort, M.T.C. *ACS Chemical Biology* **2020**, *15*, 3-16.
[3] Oerlemans, M.M.P.: Akkerman, R.; Ferrari, M.; Walvoort, M.T.C.; de Vos, P. *Journal of Functional Foods* **2021**, *76*, 104289.
[4] Ferrari, M.; Hameleers, L.; Stuart, M.C.A.; Oerlemans, M.M.P.; de Vos, P.; Jurak, E.; Walvoort, M.T.C. *Carbohydrate Polymers* **2022**, *278*, 118913.

Max Crispin

Viral glycosylation: from HIV to SARS-CoV2

Vaccine development against envelope viruses is focused on the envelope attachment glycoprotein(s) mediating cell entry and membrane fusion. The extensive glycosylation of these viral proteins is am important consideration in vaccine design. In this talk, I will discuss the use of mass spectrometry in mapping of the glycan-processing states across viral spikes, the impact of glycan density on glycan structure, and discuss the extent to which recombinant mimetic recapitulate viral glycosylation. I will compare the glycan structures arising across a panel of viruses including influenza virus, Lassa fever, HIV-1 and SARS-CoV2, and discuss the implications in viral immune evasion and vaccine design.



Mucin O-glycosylation cell-based display

Christian Büll[1,2]

[1]Department of Biomolecular Chemistry, Institute for Molecules and Materials, Science Faculty, Radboud University, Nijmegen
[2]Copenhagen Center for Glycomics, Departments of Cellular and Molecular Medicine, Faculty of Health Sciences, University of Copenhagen, Copenhagen

The diverse repertoire of human O-glycans is generated through eleven distinct glycosylation pathways. These are initiated by the transfer of GalNAc, GlcNAc, Fuc, Gal, Glc, Man, or Xyl to the oxygen of serine, threonine, or tyrosine by pathway-specific glycosyltransferases. Next to GlcNAc-type O-glycosylation (>5000 proteins), O-GalNAc-type O-glycosylation is highly abundant and can be found on >3000 proteins including the heavily O-glycosylated mucins that line the epithelial surfaces. Mucins can be expressed in membrane-bound form (for example MUC1) or secreted, gel-like form (for example MUC2). The dense O-glycosylation of mucins derives from their serine/threonine-rich tandem repeat domains. The mucin tandem repeats create rich O-glycan patterns that may encode biological information that is interpreted by the microbiome and the immune system and their numerous glycan-binding proteins, glycoside hydrolases, and proteases. Due to the tremendous size of endogenous mucins and difficulties of isolating pure material with defined glycosylation from human anatomic sites, studying molecular mucin interactions is highly challenging. Using cell-based glycan arrays, libraries of stable genetically engineered human cell lines with combinatorial knock-out and knock-in of glycosyltransferases, defined GalNAc-type O-glycans can be displayed in the natural glycan context of the cell. Expression of mucin tandem repeat domains in these glycoengineered cells enables production of membrane-bound and secreted mucin reporters with defined O-glycans. This platform is used to resemble the molecular patterns created by mucin O-glycans and to dissect how they are recognized by the microbiome and immune system. I will provide a brief overview of the diverse human O-glycosylation pathways with focus on GalNAc/mucin-type O-glycosylation. Furthermore, I will present how cell-based glycan arrays can be applied for the display of mucin reporters with defined O-GalNAc-type glycans and provide examples of molecular interactions of mucin O-glycan patterns with endogenous glycan-binding proteins and microbial mucinases.

Plasma glycoproteomics for patient diagnostics and delineation of disease glycobiology

Hans Wessels

The blood plasma glycoproteome holds great potential for biomarker discovery since abnormal glycomes have been reported for numerous genetic and acquired human diseases. Evidence is emerging that N-glycosylation may change in a protein- and site-specific manner in human disease which underlines the potential of glycopeptide analytics, or glycoproteomics, for clinical applications. Implementation of holistic glycoproteomics in routine clinical practice is now becoming a reality and opens up new opportunities to diagnose, monitor, and study disease from a site-specific glycome perspective for up to hundreds of glycoproteins from a single measurement. Focusing on congenital disorders of glycosylation, I will present on our work to implement plasma glycoproteomics in patientcare and (pre)clinical research at the Radboudumc.

Generation of glycan-specific nanobodies

Sana Khan Khilji,[1,2,8] Felix Goerdeler,[1,2,8] Kristin Frensemeier,[1,2] David Warschkau,[1,2] Jost Lühle,[1,2] Zeinab Fandi,[1] Falko Schirmeister,[1] Zhuo Angel Chen,[3] Onur Turak,[4] Alvaro Mallagaray,[4] Stefan Boerno,[5] Bernd Timmermann,[5] Juri Rappsilber,[3,6] Peter H. Seeberger,[1,2,7*] and Oren Moscovitz[1,7,*]

[1]Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, 14476 Potsdam, Germany

[2]Institute of Chemistry and Biochemistry, Freie Universität Berlin, 14195 Berlin, Germany

[3]Technische Universitat Berlin, Chair of Bioanalytics, 10623 Berlin, Germany

[4] Institute of Chemistry and Metabolomics, Center of Structural and Cell Biology in Medicine (CSCM), University of Lübeck, 23562 Lüubeck,

[5]Sequencing Core Facility, Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany

[6]Wellcome Centre for Cell Biology, University of Edinburgh, EH9 3BF Edinburgh, UK

[7] These authors contributed equally

ABSTRACT

The development of antibodies that target specific glycan structures on cancer cells or human pathogens poses a significant challenge due to the immense complexity of naturally occurring glycans. Automated glycan assembly enables the production of structurally homogeneous glycans in amounts that are difficult to derive from natural sources. Nanobodies (Nbs) are the smallest antigen-binding domains of heavy chain-only antibodies (hcAbs) found in camelids. To date, the development of glycan-specific Nbs using synthetic glycans has not been reported. By using defined synthetic glycans for alpaca immunization we prove the formation of glycan-specific hcAbs. Next, we identified, isolated, and produced Nb specific for the tumor-associated carbohydrate antigen Globo-H. The Nb binds the terminal fucose of Globo-H and recognizes synthetic Globo-H in solution and native Globo-H on breast cancer cells with high specificity. These results demonstrate the potential of our approach for generating glycan-targeting Nbs to be used in biomedical and biotechnological applications.

Glycans as a tool to reduce off-target epitopes on HIV-1 Env trimers

Ronald Derking[1], Kwinten Sliepen[1], Joel D. Allen[2], Kirsten Verheul[1], Alba Torrents de la Pena[1], Anna-Janina Behrens, Niki Bol[1], Noor Stouthamer[1,2], John P. Moore[3], Max Crispin[2], Rogier W. Sanders[1,3]

[1] Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands. [2] Biological Sciences, University of Southampton, Southampton, UK. [3] Weill Medical College of Cornell University, New York, New York, USA.
This work was supported by grants from the NIH (HIVRAD), EU (H2020; EAVI2020), AIDS Fonds, Bill and Melinda Gates Foundation.

Abstract

The HIV-1 envelope glycoprotein trimer (Env) mediates viral entry into target cells and is the main target for neutralizing antibodies (NAbs) and vaccine design efforts. The Env trimer is heavily glycosylated with *N*-linked glycans comprising ~50% of its mass. *N*-linked glycans are attached to asparagines by oligosaccharyltransferase (OST) in the amino acid sequon NxT/S, where x can be any amino acid except P. Env and Env-based vaccines, including soluble SOSIP Env trimers, can have holes in the glycan shield that result in the elicitation of strain-specific NAbs or non-NAbs. These glycan holes can stem from two different sources: 1) absence of conserved potential *N*-linked glycosylation sites (PNGS) from a particular isolate and 2) underoccupancy of a PNGS. Soluble recombinant Env trimers contain a third hole at the trimer base that is created by the removal of the transmembrane domain of Env. Immunization studies indicate that responses towards these holes are immunodominant but are dead-end path and immune-silencing these holes is highly desirable. Here, we implemented a number of different strategies to remove these glycan holes by introducing missing PNGS, using a sequon engineering strategy to enhance PNGS occupancy and limit the exposure of the trimer base by using PASylation and the introduction of a well-placed PNGS. We expect that thus reducing off-target immune responses will improve the performance of Env immunogens aimed at inducing bNAbs.

Deciphering and targeting GD2 ganglioside *O*-acetylation pathways in neuroectoderm-derived cancers

Angélina Kasprowicz[1,2], Sumeyye Cavdarli[3], Christine Bal[4], Tiziano Ingegnere[4], Nicolas Jonckheere[4], Jean-Marc Le Doussal[2], Samuel Meignan[4], Philippe Delannoy[1], Sophie Groux-Degroote[1]

[1] Univ Lille, CNRS, UMR 8576-UGSF- Unité de Glycosylation Structurale et Fonctionnelle, 59655 Villeneuve d'Ascq, France

[2] OGD2Pharma, Institut de Recherche en Santé de l'Université de Nantes, 44007 Nantes, France

[3] Laboratoire de Protéomique, Réponse Inflammatoire et Spectrométrie de Masse (PRISM), Inserm, U1192, 59000 Lille, France

[4] Univ Lille, CNRS, Inserm, CHU Lille, Institut de recherche contre le cancer de Lille, UMR9020 – UMR-S 1277 – Canther – Cancer Heterogeneity, Plasticity and Resistance to Therapies, 59000 Lille, France

The *O*-acetylated form of GD2, almost exclusively expressed in cancerous tissues, is considered to be a promising therapeutic target for neuroectoderm-derived tumors. Our recent data have shown that 9-*O*-acetylated GD2 (9-*O*AcGD2) is the major *O*-acetylated ganglioside species in breast cancer cells but the mechanism underlying GD2 *O*-acetylation remained unclear. In 2015, Baumann *et al.* proposed that Cas 1 domain containing 1 (CASD1), which is the only known human sialyl-*O*-acetyltransferase, plays a role in GD3 *O*-acetylation[1] but the mechanism of GD2 *O*-acetylation remains poorly understood. We analyzed the possible role of CASD1 in GD2 *O*-acetylation in breast cancer (BC) cells, using triple negative SUM159PT cells that endogenously express *O*AcGD2 as a model. The modulation of CASD1 expression using transient transfection strategies in triple negative breast cancer cells provided interesting insights into the role of CASD1 in *O*AcGD2 and *O*AcGD3 biosynthesis, and it highlights the importance of further studies on *O*-acetylation mechanisms[2]. Consequently, in order to identify additional genes involved in OAcGD2 biosynthesis in BC, we performed a kinome/phosphatome siRNA screen and identified genes that up- or down-regulate OAcGD2 expression. *CERK* (ceramide kinase gene) was selected for further studies among genes of interest. Ceramide kinase, the enzyme that synthesizes ceramide-1-phosphate from ceramide, has a direct link with glycosphingolipid biosynthesis that starts with the conversion of ceramide into glucosylceramide. In addition, *CERK* inhibition by siRNA induced a significant increase of OAcGD2 expression in BC cells in the RNAi screen. We confirmed the effect of *CERK* inhibition on OAcGD2 expression using pharmacological inhibitors and individual siRNA in different BC cell lines, and studied the impact of *CERK* inhibition on the malignant properties of BC cells. Our data highlight the potential of increasing the efficacy of OAcGD2-targeted immunotherapy with compounds such as *CERK* inhibitors that increase OAcGD2 expression.

1. Baumann, A.-M. T. *et al.* 9-O-Acetylation of sialic acids is catalysed by CASD1 via a covalent acetyl-enzyme intermediate. *Nat. Commun.* **6**, 7673 (2015).

2. Cavdarli, S. *et al.* Role of Sialyl-O-Acetyltransferase CASD1 on GD2 Ganglioside O-Acetylation in Breast Cancer Cells. *Cells* **10**, 1468 (2021).

TOWARDS FULL CONTROL OVER PROTEIN GLYCOSYLATION: *IN-VITRO* GLYCOENGINEERING OF INFLUENZA A VIRUS HEMAGGLUTININ AND SARS-CoV-2 SPIKE PROTEIN

Mariana Juárez [1], Annabell Darr [1], Julia Volckmar[2], Dunja Bruder [2,3], Thomas Rexer [1], Udo Reichl [1,4]

[1] Max Planck Institute for Dynamics of Complex Technical Systems, Bioprocess Engineering, Sandtorstr. 1, 39106 Magdeburg, Germany
[2] Infection Immunology, Institute of Medical Microbiology, Infection Prevention and Control, Health Campus Immunology, Infectiology and Inflammation Otto-von-Guericke University Magdeburg, Germany
[3] Immune Regulation Group, Helmholtz Centre for Infection Research, Braunschweig, Germany
[4] Otto-von-Guericke University Magdeburg, Chair of Bioprocess Engineering, Magdeburg, Germany

The baculovirus-insect cell expression system is readily utilized to produce viral glycoproteins for research and pharmaceutical applications. This includes, for instance, subunit vaccines and vaccine candidates against Influenza and SARS-CoV-2 infections. However, with mainly complex-type *N*-glycans attached, the glycoforms of recombinant proteins derived from this expression system are inherently different from mammalian cell-derived glycoforms and the impact of these differences on the immunogenicity has hardly been studied in detail [1,2]. This applies also to the Influenza A virus hemagglutinin (HA) and the SARS-CoV-2 spike protein, which are antigen targets of nearly all licensed vaccines and vaccine candidates including virus like particles and subunit vaccines.

To generate specific homogeneous glycoforms, we have developed an *in-vitro* glycoengineering platform consisting of recombinant Leloir glycosyltransferases expressed in *E.coli*. To demonstrate the applicability of the platform, we have extended paucimannose glycans of Sf9-derived influenza A virus HA (H10N08) to obtain the terminal structure Galα1-3Galβ which corresponds to the α-Gal epitope. Since the anti-Gal antibody is naturally found in humans and constitutes approximately 1% of immunoglobulin G, the α-Gal epitope can be potentially used as a target for developing glycan-based vaccines against different diseases [3].

Furthermore, to demonstrate the practical implementation of the platform, his-tagged High Five-derived SARS-CoV-2 spike protein was immobilized on Ni-NTA magnetic agarose beads for *in-vitro* glycoengineering utilizing a set of soluble glycosyltransferases. Galactosylated glycans with Galβ1-4 termina were identified by xCGE-LIF analysis with almost the same proportion compared to the *in-vitro* glycosylated spike obtained without the immobilization protocol. Thus, the possibility to perform the reactions for the *in-vitro* glycoengineering with the immobilized target protein was demonstrated.

The *in-vitro* glycoengineering approach established can be used to efficiently modify a wide range of *N*-glycans on vaccine candidates and therapeutic proteins. To commercialize our technology, we have acquired funding from the EXIST-research transfer program of the Federal Ministry for Economic Affairs and Energy. The project aims to establish a spin-off, eversyn, co-founded by the Max Planck Society by 2024.

Keywords: *In-vitro* glycoengineering, α-Gal epitope, vaccine design, SARS-CoV-2 spike, Influenza A virus H1N1 hemagglutinin, viral envelope proteins.

References

1. Palomares, L.A., *et al.*, *Glycobiotechnology of the Insect Cell-Baculovirus Expression System Technology*. Adv Biochem Eng Biotechnol. 2021. 175:71-92.
2. Schön, K., *et al.*, *Impact of Protein Glycosylation on the Design of Viral Vaccines*. Adv Biochem Eng Biotechnol. 2021;175:319-354.
3. Galili, U., *Amplifying immunogenicity of prospective Covid-19 vaccines by glycoengineering the coronavirus glycan-shield to present α-gal epitopes*. Vaccine. 2020. 38(42):6487-6499.

Mapping the antibody repertoire to *Staphylococcus aureus* wall teichoic acid reveals a protective role for IgM during invasive infection

Astrid Hendriks[1], Priscilla Kerkman[2], Sara Ali[3], Meri R.J. Varkila[4], Jelle Haitsma-Mulier[4], Carla J.C. de Haas[2], Piet C. Aerts[2], Olaf L. Cremer[5], Marc J.M. Bonten[2,4], Suzan H.M. Rooijakkers[2], Jos A.G. van Strijp[2], Jeroen D.C. Codée[3] and Nina M. van Sorge[1,6,7,#]

[1]Department of Medical Microbiology and Infection Prevention, Amsterdam UMC, location University of Amsterdam, Amsterdam, the Netherlands.
[2]Department of Medical Microbiology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
[3]Leiden Institute of Chemistry, Leiden University, Leiden, the Netherlands
[4]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
[5]Department of Intensive Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
[6]Netherlands Reference Center for Bacterial Meningitis, Amsterdam UMC, location AMC, Amsterdam, the Netherlands
[7]Amsterdam Institute for Infection and Immunity, Infectious Diseases, Amsterdam, The Netherlands

Staphylococcus aureus is one of the leading causes of hospital-acquired infections with high overall mortality. Pre-existing immunity to *S. aureus* is omnipresent among healthy individuals due to natural exposure, although this is not always sufficient to protect from (re-)infection. Antibodies are believed to play a key role in bacterial killing through antibody opsonization and complement activation, which induces subsequent bacterial uptake as well as neutrophil recruitment. However, the exact correlates of protection remain to be fully elucidated. A large proportion of the anti-*S. aureus* antibody pool is directed against Wall Teichoic Acid (WTA), an abundant cell wall glycopolymer, which shows structural variation through glycosylation with *N*-acetylglucosamine (GlcNAc). Overall, three WTA glycotypes are currently distinguished and IgG antibody responses reactive with these different WTA glycotypes can be detected in plasma from healthy individuals. How these antibody profiles are affected during invasive *S. aureus* infection is not known but may help to identify protective responses. Here, we analyzed the antibody repertoire to *S. aureus* WTA glycotypes in plasma from healthy individuals (n=31) and ICU patients with culture-confirmed *S. aureus* bacteremia (n=36) using a recently developed bead assay with glycosylated synthetic WTA fragments. Robust IgM responses to all three WTA modifications were detected in 30 out-of-31 healthy individuals. In contrast, WTA-specific IgM responses were significantly decreased or even non-detectable in *S. aureus* bacteremia patients, whereas no differences in IgG2 responses were observed. Using longitudinal samples, we observed limited variation of WTA-specific IgM responses during the course of infection. Furthermore, the absence of IgM WTA-reactivity was associated with disease

mortality, which resulted in impaired antibody-mediated *in vitro* complement activation on live *S. aureus* bacteria. This study supports the existence of a broad antibody repertoire to *S. aureus* WTA glycotypes, and proposes a protective role of WTA-specific IgM against invasive *S. aureus* infections.

Glycan shielding on Dengue and Zika virus envelopes against broadly neutralizing E-dimer epitope (EDE) antibodies revealed by mass spectrometry

Tatiana M. Shamorkina[1], Juthathip Mongkolsapaya[2], Wanwisa Dejnirattisai[2], Arvind Sharma[3], Felix A. Rey[3], Gavin Screaton[4], Joost Snijder[1]

[1]Biomolecular Mass Spectrometry and Proteomics, Department of Chemistry, Faculty of Science, University of Utrecht, Utrecht, The Netherlands

[2]Wellcome Center for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, UK

[3]Institut Pasteur, Structural Virology Unit, CNRS UMR3569, Paris, France

[4]Division of Medical Sciences, University of Oxford, Oxford, UK

Background: Annually, around 100 million clinically manifested cases of Dengue fever in >100 countries are caused by four Dengue virus (DENV) serotypes, for which no effective vaccine is available. To date, cross-protective immunity against all four serotypes is not readily achieved. Recently, potent cross-neutralizing antibodies have been isolated and characterized that recognize the so-called envelope dimer epitope, EDE, on the DENV envelope glycoprotein. Additionally, EDE-antibodies effectively cross-neutralize closely related Zika virus. The structure of EDE-antibody in complex with the envelope antigen shows the presence of N-glycans at the antibody-binding site. Although, the epitope is structurally conserved, the N-linked glycosylation can be remarkably diverse. Here, we use a hybrid mass spectrometry approach to characterize N-linked glycosylation of recombinant E-subunits in ZIKV and 3 DENV serotypes, and investigate the role specific glycoforms play in recognition by the EDE antibodies.

Methods: We used soluble insect and mammalian derived E-ectodomains of DENV1-4 and ZIKV. The E-dimers were analyzed by LC-MS/MS with EThcD fragmentation scheme. Using MS as a readout, we probe which site-specific glycoforms are preferentially bound by EDE antibodies through immunoaffinity enrichment. We use virus neutralization assays to test our mass spectrometry generated glycoproteomics data.

Results/Conclusions: In total, we quantified over 30 glycoforms on insect derived E-dimers and over 100 glycoforms on mammalian derived E-dimers. We demonstrate that EDE-antibodies preferentially bind DENV-2 and ZIKV E subunits, decorated with truncated chitobiose structures at N67 and N154 sites respectively, indicative of strong *N*-glycan shielding. The latter is supported by our neutralization assays with DENV viruses lacking glycosites by mutagenesis. Our findings can play a positive role in future subunit vaccine design.

The use of mass spectrometry and glycogenomics for the dissection of the human *O*-glycome

Noortje de Haan[a], Yoshiki Narimatsu[a], Ming Song[a], Sergey Y Vakhrushev[a], Hans H Wandall[a]

[a] Copenhagen Center for Glycomics, University of Copenhagen, Copenhagen, Denmark

The examination of *O*-glycan structures in cells and biological systems has been challenged by the absence of an enzyme for the unbiased release of *O*-glycans from their protein carrier as well as by the immense abundance of *O*-glycan structural isomers [1]. While chemical release strategies based on reductive β-elimination have been indispensable for the analysis of free *O*-glycans by mass spectrometry (MS), the inherent protection of the reducing end *via* reduction prevents the functionalization of the glycans required for a diverse array of analytical techniques. While recent developments offer possibilities for more broadly applicable analysis of free *O*-glycans [2], it remains challenging to apply such methods on complex samples, to characterize potential *O*-glycan isomers and to integrate the analysis in a high-throughput setup.

To address these issues, we further developed a minimally destructive, non-reductive release of *O*-glycans from proteins in tissue homogenates and cell lysates [3]. The method allows multiplexed sample preparation in a 96-well format as well as the sequential release of *N*- and *O*-glycans from the same sample. Uniform labeling of the glycan reducing end enables efficient C18 liquid chromatography (LC)-MS analysis, using a standard proteomics set-up and featuring glycan isomer separation.

Application of the method on an array of glyco-engineered human cell lines, e.g., EOGT, C1GALT1, GCNT1 and sialyltransferase knock outs, resulted in the structural annotation of *O*-glycans derived from human cells and tissue, including the annotation of initiating monosaccharides and *O*-GalNAc core elongation. Using keratinocytes as model system, we found a wide variety of *O*-glycan structures, including *O*-fucose, *O*-glucose and *O*-GalNAc glycosylation, with the latter carrying both elongated core1 and core2 structures and varying numbers of fucoses and sialic acids.

The method will be applied on human tissue and disease models to characterize the potential change of specific glycan structures during differentiation and disease.

[1] Čaval, T., De Haan, N., Konstantinidi, A., & Vakhrushev, S. Y. (2021). Quantitative characterization of O-GalNAc glycosylation. Current Opinion in Structural Biology, 68, 135-141.

[2] Kameyama, A., Tin, W. W. T., Toyoda, M., & Sakaguchi, M. (2019). A practical method of liberating O-linked glycans from glycoproteins using hydroxylamine and an organic superbase. Biochemical and biophysical research communications, 513(1), 186-192.

[3] De Haan N., Narimatsu Y., Koed Møller Aasted M., Larsen I. S. B., Marinova I. N., Dabelsteen S., Vakhrushev S. Y., Wandall H. H. (2022). In-Depth Profiling of O-Glycan Isomers in Human Cells Using C18 Nanoliquid Chromatography-Mass Spectrometry and Glycogenomics. Anal Chem. 94(10):4343-4351.

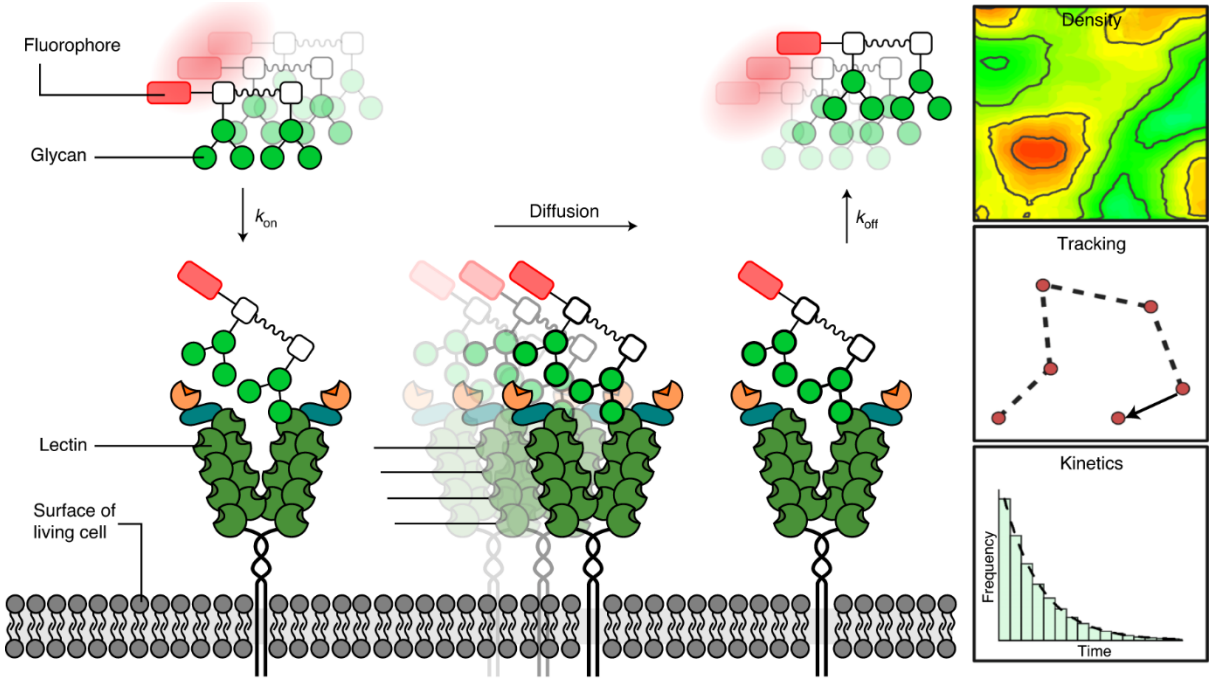
Glyco-PAINT: quantifying glycan-lectin binding kinetics at the single molecule level on living cells

Roger Riera, Tim P. Hogervorst, Ward Doelman, Yan Ni, Silvia Pujals, Evangelia Bolli, Jeroen D. C. Codée, Sander I. van Kasteren & Lorenzo Albertazzi

Nat. Chem. Biol. **2021**, *7*, 1281–1288

While the interaction between cell-membrane bound lectins and their carbohydrate ligands are crucial to many aspects of glycobiology, the study of these interactions is hindered by the typically low affinity of the interacting partners. Recently, we have developed a novel technique to directly measure these interaction on the cell-surface using single molecule microscopy, which we dubbed glyco-PAINT. Our approach is based on point accumulation in nanoscale tomography (PAINT) super-resolution microscopy, where the weak and reversible binding enables direct detection of the interaction on a single-molecule level. To prove the feasibility of this approach, we chose the mannose receptor (MR), an important immune cell receptor for the uptake of glycosylated antigens, as our model lectin. The MR has several C-type lectin (CTL) domains that bind to mannosides, as well as a cysteine rich domain (CRD) that binds sulfated sugars.

A library of synthetic, fluorescently labeled, multivalent carbohydrate probes was produced; the probes contained different configurations and valences of mannosides or 4-sulfo-GalNAc. Using PAINT microscopy, the relative k_{on} and k_{off} for the interaction with the mannose receptor could be determined for each, and structure-activity relationships for the different sugars could be established. Furthermore, tracking of the carbohydrate-lectin complex also enables the measurement of the diffusion coefficient of the receptor. Uptake of the fluorescent glycoclusters was determined by FACS, and a strong correlation with the binding parameters as determined by glyco-PAINT was established. In conclusion, glyco-PAINT enables, for the first time, direct measurement of the kinetics of glycan-lectin interactions on the surface of a living cell.



In-vitro multi-enzyme cascades for the synthesis of uridine nucleotides sugars

Tuan Hoang Son[1], Reza Mahour[1,*], Alberto Alcalá[1], Thomas F. T. Rexer[1], Udo Reichl[1,2]

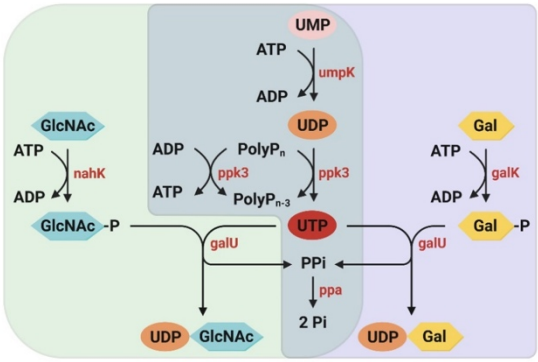
[1] Max-Planck-Institute for Dynamics of Complex Technical Systems, Bioprocess Engineering, Sandtorstr. 1, 39108 Magdeburg, Germany
[2] Otto-von-Guericke University Magdeburg, Chair of Bioprocess Engineering, 39106 Magdeburg, Germany
[*] now at c-LEcta GmbH, Leipzig, Germany

Abstract

The high price and limited availability of nucleotide sugars is impeding the enzymatic synthesis of valuable functional oligosaccharides and glycoconjugates such as human milk oligosaccharides or glycoproteins and glycolipids (1; 2). Through innovative reaction engineering, we have developed *in vitro* multi-enzyme cascades to produce nucleotide sugars such as, UDP-galactose (UDP-Gal), UDP-*N*-Acetylglucosamine (UDP-GlcNAc) and UDP-*N*-Acetylgalactosamine (UDP-GalNAc) from inexpensive precursors (3; 4). All enzymes are recombinantly produced in *E. coli* in their soluble and active form.

Using the cascades shown above, UDP-GlcNAc and UDP-Gal can be produced from the inexpensive substrates UMP, ATP, polyphosphate, Gal and GlcNAc, respectively. To reduce costs, ATP is *in situ* regenerated from polyphosphate.

As an example, UDP-GlcNAc can be produced using affinity purified enzymes with a conversion yield of 91% with respect to 50,3 mM UMP and a final product concentration of 29.8 g/L in a batch time of 24 h hours and a volume of 5 mL. The high conversion yield enabled simple purification using Amicon filters with cut-off of 10 kDa resulting in a purity of at least with only polyphosphate as major impurity. To further increase purity anion exchange chromatography is applied. Therefore, a strong anion exchange resin designed for preparative protein purification can be used. A determined dynamic binding capacity of approximately 24 mg_{UTP}/mL_{resin} allows a purification in milligram scale per passage.



To commercialise nucleotide sugars and selected glyco structures, we have acquired funding from the EXIST-research transfer programme. The project called **SynGlyco** started in February 2022 and aims to establish a spin-off company by 2024.

1. **Faijes, M., et al.** Enzymatic and cell factory approaches to the production of human milk oligosaccharides. s.l. : Biotechnology Advances, 2019, pp. 667-697.

2. **Figueroa-Lozano S, de Vos P.** Relationship Between Oligosaccharides and Glycoconjugates Content in Human Milk and the Development of the Gut Barrier. Compr Rev Food Sci Food Saf. 2019, pp. 121-139.

3. **Mahour, R., et al.** Cell-Free Multi-Enzyme Synthesis and Purification of Uridine Diphosphate Galactose . s.l. : ChemBioChem, 2022.

4. —. Establishment of a five-enzyme cell-free cascade for the synthesis of uridine diphosphate N-acetylglucosamine. s.l. : Journal of Biotechnology, 2018, pp. 120-129.

5. —. Multi-enzyme cascades for the in vitro synthesis of guanosine diphosphate L-fucose. s.l. : ChemCatChem, 2021, pp. 1-10.

Sam J. Moons[1], **Daniël L.A.H. Hornikx**[2], Christian Büll[2,3], Thomas J. Boltje[1]

[1]Cluster for Molecular Chemistry, Institute for Molecules and Materials, Radboud University Nijmegen, Nijmegen, The Netherlands
[2]Department of Biomolecular Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen, The Netherlands
[3]Copenhagen Center for Glycomics, Department of Cellular and Molecular Medicine, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Light-controlled spatiotemporal desialylation with a photoactivatable sialyltransferase inhibitor

Fluorinated sialic acids are potent metabolic inhibitors of the sialyltransferases and effectively block sialic acid capping of glycans in mammalian cells and bacteria (1-3). These sialyltransferase inhibitors are highly useful tools to probe the contribution of sialic acids in cellular processes and glycan interactions at the molecular level. Moreover, sialyltransferase inhibitors have been reported to reduce metastasis and to increase immunogenicity of tumors in mouse models and can potentially be applied to reduce viral and bacterial infection that relies on sialoglycans (3-5). These studies strongly advocate that inhibition of sialylation with chemically pure small molecule inhibitors of sialyltransferases can have therapeutic effects in cancer and infection. The major challenge for the therapeutic application of sialyltransferase inhibitors is to achieve local activity in target tissues such as tumors or infected areas, because systemic deletion of sialic acids can result in adverse effects due to their physiological importance (5). To enable selective and local activity of a known potent sialic acid-based sialyltransferase inhibitor (SiaFEToc), we have installed a photolabile 2-nitrobenzyl ether protecting group at the anomeric center of the sialic acid inhibitor yielding photo-SiaFEToc. The photolabile group rendered SiaFEToc inactive and no activity was found in human cell lines treated with photo-SiaFEToc. Irradiation with 365 nm UV-light cleaved the protecting group and produced the compound that is metabolized to the active inhibitor. Targeted and short irradiation with 365 nm UV-light of a cell monolayer pulsed with photo-SiaFEToc resulted in effective and local desialylation. We will present our strategy for development of this photoactivatable sialyltransferase inhibitor and demonstrate its use for controlled local desialylation in mammalian cell cultures. Potentially, this approach can be applied for local inhibition of sialoglycan biosynthesis in superficial tumors penetrable by UV light and omit the adverse effects of systemic inhibition of sialylation.

References

1. Rillahan et al. Global metabolic inhibitors of sialyl- and fucosyltransferases remodel the glycome. *Nature Chemical Biology*. 2012. 8(7):661-668
2. Heise et al. Sialyltransferase inhibitors based on C-5 modified fluorinated sialic acids. *Journal of Medicinal Chemistry*. 2019. 62(2):1014-1021
3. Moons et al. The structure – activity relationship of sialic acid glycomimetics. *ACS Chemical Biology*. 2022. 17(3):590-597
4. Büll et al. Sialic acid blockade suppresses tumor growth by enhancing T cell-mediated tumor immunity. *Cancer Research*. 2018. 78(13):3574-3588
5. Heise et al. Selective inhibition of sialic acid-based molecular mimicry in Haemophilus influenzae abrogates serum resistance. *Cell Chemical Biology*. 2018. 25(10):1279-1285

Why are Cys domains of mucins not C-mannosylated?

Marco Albers[1], Jonas Kaynert[1], Andreas Pich[2], Hans Bakker[1]

[1]Institute of Clinical Biochemistry, Hannover Medical School, 30625 Hannover, Germany

[2]Institute for Toxicology, Hannover Medical School, 30625 Hannover, Germany

Abstract:

Mucins are the major component of the mucus, the first biological barrier at the surface of secretory epithelia. Mucins are high molecular weight glycoproteins and they occur secreted or membrane-bound. Serine-, threonine- and proline-rich regions are highly O-glycosylated. These S/T/P regions are interrupted by Cys domains (CysDs), which are small domains with 8 conserved cysteine residues. CysDs contain conserved WxxW motifs, the consensus for C-mannosylation. Therefore, it has been hypothesized that CysDs are C-mannosylated. However, previous studies could not proof this. Hence, we investigated the potential C-mannosylation on mucin CysDs by mass spectrometry. We recombinantly expressed CysD5 of human MUC5AC in CHO cells. MUC5AC is a secreted, gel-forming mucin. We purified His-tagged CysD5 with Ni-NTA-chromatography and analyzed digested peptides by LC-MS. MS analysis revealed that this CysD is not C-mannosylated, even if the protein is retained to the ER.

However, we showed that CysD are C-mannosylated if conserved cysteine-residues are mutated. The mutated CysDs were almost fully C-mannosylated. These cysteine-residues form intramolecular disulfide bridges. We compared these results to the CysD from cartilage intermediate layer protein 1 (CILP1). This CysD contains the conserved WxxW motif but not the mentioned cysteine-residues. In CHO cells, CILP1 CysD is C-mannosylated.

Our experiments suggest that the motif of mucin CysDs is a potential substrate for C-mannosyltransferases, but cysteine-residues hinder C-mannosylation. We propose that formation of intramolecular disulfide bridges or co-translational binding of chaperones to adjacent cysteine-residues prevents C-mannosylation.

MUC13: a fine, sweet, line between epithelial maintenance and tumorigenesis

Daphne A.C. Stapels, Paul J. Besseling, Noortje Ijssennagger, Xinyue Li, Nancy M.C. Bleumink-Pluym, Jos P.M. van Putten and Karin Strijbis

Mammalian mucosal surfaces consist of a mucus layer composed of secreted mucins and underlying epithelial cells that express cell-attached mucins. Both types of mucins are extensively O-glycosylated. They protect against damages from outside like bacterial invasion. In addition, cell-attached mucins carry EGF-like domains, a transmembrane domain and a cytoplasmic tail that can contribute to intracellular epithelial signaling.

MUC13 is the most conserved, ancestral transmembrane mucin. It localises to tight junctions in the lateral membrane, and to the apical side of epithelial cells. MUC13 is the smallest transmembrane mucin, thereby forming a fine line of defence on the epithelial cells. In addition, MUC13 is known to contribute to cancerous cell behaviour. Interestingly, single-cell RNA sequencing data showed ubiquitous expression of MUC13 by various epithelial cell types along the entire intestinal tract. Therefore we hypothesise that MUC13 has an essential, broad role in gut epithelial maintenance that might be exploited by colorectal cancer (CRC) cells.

To study MUC13, we first generated appropriate tools. We designed a CRISPR/Cas9 knockout strategy, a MUC13-GFP plasmid for inducible overexpression, and generated a novel antibody against the extracellular domain of MUC13. Removal of MUC13 from the fast-growing, migratory colorectal cell line HRT18 greatly reduced cell proliferation and migration speed. During migration, MUC13 localised to the leading edge and contributed to collective migration and stabilisation of E-cadherin connective structures between adjacent leader cells. MUC13 knockout cells displayed altered epidermal growth factor receptor (EGFR) signalling. Together, our results demonstrate that MUC13 controls proliferation, formation of cell junctions and cell migration which may in part be explained by altered EGFR signalling. These functions might contribute to barrier integrity and wound healing in healthy epithelium and promote metastases in tumour cells due to over expression of MUC13. Whether the O-glycosylation pattern of MUC13 influences its functions is still to be determined.

Exploration of functional roles of glycosphingolipids using glycosphingolipid-depleted human iPSCs and their derivatives

Julia Beimdiek and Falk F.R. Buettner
Institute of Clinical Biochemistry, Hannover Medical School, Carl-Neuberg-Str. 1,Hannover, Germany

Glycosphingolipids (GSLs) form a heterogeneous group of glycosylated membrane lipids which are involved in basic cellular processes and can be associated with several diseases[1,2]. GSLs are not essential at the level of stem cells but are required during development of multicellular organisms[3]. Knowledge about the function of individual GSLs during the process of differentiation is scarce. To gain further insights, we generated CRISPR/Cas9 knockouts (KOs) of either glucosylceramide- (UGCG) or galactosylceramide-synthase (UGT8) in human induced pluripotent stem cells (hiPSCs). Thereby the two major groups of GSLs were depleted individually enabling the dissection of group specific functions. Analysis of GSL glycosylation by capillary gel electrophoresis coupled to laser induced fluorescence detection (xCGE-LIF)[4], clearly demonstrated the functional impairment of UGCG. Initial characterization by morphology and stem cell marker expression did not reveal any anomalies in both, the *UGCG*- and *UGT8*-KO hiPSCs. Characteristic marker expression and morphology during endodermal and mesodermal differentiation was not altered in both KOs. However, during ectodermal differentiation *UGCG*-KO hiPSCs did not survive passaging. Interestingly, it was recently shown that glucosylceramide is synthesized upon neuronal activity and transported from neurons to glia through exosomes[5]. Therefore, UGCG-KO hiPSCs are ideal to examine the potential role of glucosylceramides not only at a mature stage but also at very early time points during development. To uncover potential general defects of the *UGCG*- and *UGT8*-KO hiPSCs associated with the interaction of different cell types during differentiation, we performed teratoma assays. We observed reduced formation of ectoderm in teratoma originating from *UGT8* KO cells. Comparative quantitative proteomic analysis of teratoma derived from wild type, *UGCG*- and *UGT8*-KO hiPSCs is currently ongoing. Taken together, *UGCG*- and *UGT8*-KO hiPSCs hold great potential as model systems for the identification of specific GSL functions.

[1]*Daniotti, J., Vilcaes, A., Torres Demichelis, V., Ruggiero, F. & Rodriguez-Walker, M. Glycosylation of Glycolipids in Cancer: Basis for Development of Novel Therapeutic Approaches. Frontiers in Oncology 3, doi:10.3389/fonc.2013.00306 (2013).*
[2]*Rodríguez, E., Schetters, S. T. T. & van Kooyk, Y. The tumour glyco-code as a novel immune checkpoint for immunotherapy. Nature reviews. Immunology 18, 204-211, doi:10.1038/nri.2018.3 (2018).*
[3]*Yamashita, T. et al. A vital role for glycosphingolipid synthesis during development and differentiation. Proceedings of the National Academy of Sciences of the United States of America 96, 9142-9147 (1999).*
[4]*Rossdam, C. et al. Approach for Profiling of Glycosphingolipid Glycosylation by Multiplexed Capillary Gel Electrophoresis Coupled to Laser-Induced Fluorescence Detection To Identify Cell-Surface Markers of Human Pluripotent Stem Cells and Derived Cardiomyocytes. Analytical chemistry 91, 6413-6418, doi:10.1021/acs.analchem.9b01114 (2019).*
[5]*Wang L, Lin G, Zuo Z, Li Y, Byeon SK, Pandey A, Bellen HJ. Neuronal activity induces glucosylceramide that is secreted via exosomes for lysosomal degradation in glia. Sci Adv. 2022 Jul 15;8(28):eabn3326. doi: 10.1126/sciadv.abn3326. Epub 2022 Jul 13.*

Multi-TaG: A modular platform of bioorthogonal probes for multi-modal glycoprotein analysis

Marc D. Driessen[1], Hauke Junghans[2], Ulla I. M. Gerling-Driessen[2]
[1]Heinrich Heine University Duesseldorf, Molecular Proteomics Laboratory, Universitätsstraße 1, 40225 Düsseldorf, Germany
[2]Heinrich Heine University Duesseldorf, Institute of Organic and Macromolecular Chemistry, Universitätsstraße 1, 40225 Düsseldorf, Germany; ulla.gerling-driessen@hhu.de

Congenital Disorders of Glycosylation (CDGs) are gene mutations that impair the function of enzymes involved in the glycoprotein biosynthesis. Previously, we investigated NGLY1 deficiency, a CDG caused by loss-of-function mutations in the gene encoding for the enzyme N-Glycanase 1, which removes N-Glycans from misfolded glycoproteins.[1] Linking misglycosylated proteins in CDGs to impaired biological functions and clinical phenotypes, requires an untargeted analysis of the glycoproteome. The heterogeneity and diversity of glycoproteins makes a detailed analysis very challenging, demanding for new tools and strategies.

Here, we present a strategy that is based on metabolic oligosaccharide engineering of the glycoproteome and subsequent covalent tagging of the glycan structures with analytical probes using bioorthogonal chemistry. We developed a platform of bioorthogonal probes that allow the combination of multiple experiments by using only one tag that is attached to the glycoproteins. Our approach uses established principles of solid phase peptide synthesis to combine multiple functional building blocks (fluorophores, cross linkers, cleavable linkers, affinity units and isotopic labels etc.) on a solid support. The probe design contains a central glycine motif in different isotopic versions (e.g., D_2, ^{13}C, ^{15}N), which provides the opportunity to equip every probe with an inherent label to be utilized for intact, quantitative glycoproteomics. Using a special linker on the solid support, our synthesis strategy allows probe release and Cterminal functionalization with any bioorthogonal handle in one step.

We show that our library of multi-functional probes allows smart workflows and concerted analysis of glycoproteins with different methods (i.e., imaging, isolation, targeted intact and quantitative glycoproteomics). While established bioorthogonal probes can be limited with regard to flexibility in either experimental read-out options or applicable bioorthogonal attachment chemistry, our platform overcomes both of these limitations and provides a powerful tool to analyze aberrantly glycosylated proteins in CDGs and other diseases.

[1] Tomlin, F. M., Gerling-Driessen, U. I. M., Liu, Y. C., Flynn, R. A., Vangala, J. R., Lentz, C. S., Clauder-Muenster, S., Jakob, P., Mueller, W. F., Ordonez-Rueda, D., Paulsen, M., Matsui, N., Foley, D., Rafalko, A., Suzuki, T., Bogyo, M., Steinmetz, L. M., Radhakrishnan, S. K., and Bertozzi, C. R. Inhibition of NGLY1 Inactivates the Transcription Factor Nrf1 and Potentiates Proteasome Inhibitor Cytotoxicity, ACS Cent. Sci. 2017, 3, 1143-1155.

Deficiency of Pseudoautosomal DHRSX Sheds a New Light on Dolichol Metabolism

Charlotte Althof

N-Glycosylation is among the most important post-translational modifications of proteins in eukaryotes. In Congenital Disorders of Glycosylation (CDG), mutations in genes involved in performing or controlling glycosylation lead to incorrect protein modifications and severe disease. Several CDG have been described that are caused by defects in the metabolism of the isoprenoid dolichol. Dolichol is the precursor lipid of dolichol-phosphate which acts as the ER membrane-bound lipid carrier for oligosaccharides aiding in their transfer onto a nascent protein. We describe here DHRSX-CDG, a disorder leading to neurodevelopmental, ophthalmological, hepatic and dysmorphic features. In patient cells and cell models, abnormalities in the balance of several isoprenoid species suggest that the metabolism of these essential lipids is more complex than previously understood, and point towards DHRSX as a missing enzyme in this pathway. DHRSX-CDG is the first described truly recessive disorder caused by variants in the pseudoautosomal recessive regions of the X/Y chromosomes.

DECIPHERING SIALIC ACID PATHWAY REGULATION VIA IN-DEPTH MULTI-OMICS APPROACH IN TISSUE-SPECIFIC HUMAN MODELS

Merel A. Post[1], Rachel Mijdam[1,3], Melissa J. Bärenfänger[4], Raisa Veizaj[1],Fokje SM Zijlstra[2], Johan FA Pijnenborg[6], Clara DM van Karnebeek[5], Hans JCT Wessels[2], Thomas J Boltje[6], Nael Nadif Kasri[3,7], Dirk J. Lefeber[1]

1 Department of Neurology, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, Netherlands

2 Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, Netherlands

3 Department of Human Genetics, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, Netherlands

4 Division of BioAnalytical Chemistry, Amsterdam Institute for Molecular and Life Sciences, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

5 Department of Pediatric Metabolic Diseases, Emma Children's Hospital, Amsterdam University Medical Center, Amsterdam, Netherlands

Department of Synthetic Organic Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen, The Netherlands.

7 Department of Cognitive Neuroscience, Radboud University Medical Center, Donders Institute for Brain, Cognition and Behavior, Nijmegen, the Netherlands

Abstract

Sialic acid is the most abundant terminal monosaccharide on glycan structures of glycoproteins, glycolipids and gangliosides. Due to their abundance and electronegative charge, they have many different functions including cell-cell interactions, protein stabilization but also remodeling of mature neuronal connections. Sialic acid is involved many disorders such as Salla disease, atherosclerosis and congenital disorders of glycosylation (CDG). Mutations in sialic acid synthase gene (*NANS)* causes NANS-CDG with diverse symptoms including skeletal dysplasia, short stature and facial dysmorphisms. They have a clear neurological phenotype including neurodegeneration and intellectual disability. However, other genetic defects related to sialic acid metabolism have different phenotypes, suggesting the existence of tissue-specific mechanisms. To study the general glycosylation defects of defective NANS on glycans and glycoproteins, total plasma glycomics and glycoproteomics was performed. No clear changes were observed using glycomics. However, glycoproteomics revealed site-specific effects of *NANS* deficiency on sialylation and N-glycan branching. Furthermore, we studied sialic acid metabolism in patient fibroblasts, induced pluripotent stem cells, its derived neurons and HAP1 knockout cells. First, we characterized all metabolites involved in the sialic acid and hexosamine biosynthesis pathway in HAP1 *NANS* KO cells, patient fibroblasts and iNeurons to obtain an overview of aberrant metabolism. *NANS* KO cells, patient fibroblasts, patient derived iPSCs and iNeurons showed dramatically high levels of ManNAc-6P which is in line with enzymatic deficiency of NANS. Unexpectedly, levels of CMP-sialic acid and sialic acid were only decreased in *NANS* KO HAP1 cells. Surprisingly, UDP-HexNAc levels were clearly decreased in *NANS* KO cells and moderately decreased in patient fibroblasts, iPSCs and iNeurons. Further analysis of the metabolic pathway revealed a block in hexosamine biosynthesis pathway due to accumulating ManNAc-6P, which was confirmed by direct enzymatic assays. This revealed a novel regulatory pathway between hexosamine and sialic acid pathways and could explain branching defects observed by glycoproteomics.

Poster presentations

In alphabetical order

Poster 1

The diversity of the glycan shield of sarbecoviruses closely related to SARS-CoV-2

Joel D. Allen*[1], Dylan Ivory[1], Sophie Ge Song[2,3,4], Wan-ting He[2,3,4], Tazio Capozzola[2,3,4], Peter Yong[2,3,4], Dennis R. Burton[2,3,4,5], Raiees Andrabi[2,3,4], Max Crispin*[1]

[1] School of Biological Sciences, University of Southampton, Southampton, SO17 1BJ, UK

[2] Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA 13 92037, USA

[3] IAVI Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, CA 92037, USA

[4] Consortium for HIV/AIDS Vaccine Development (CHAVD), The Scripps Research Institute, La Jolla, CA 92037, USA

[5] Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University, Cambridge, MA 02139, USA.

The emergence of SARS-CoV-2, the causative agent of COVID-19 represents the third coronavirus in the 21st century to crossover from animals to humans and cause a severe outbreak of disease. In addition to SARS-CoV-2, there are many closely related coronaviruses circulating in bats that have potential to crossover into humans. Whilst current vaccines against SARS-CoV-2 have proven very successful at limiting severe disease and death globally, the emergence of more resistant variants gives cause for concern about the utility of these vaccines in the longer term. Furthermore, there may be additional coronavirus crossovers in the future that trigger new pandemics. Therefore, there is a strong push to develop pan-coronavirus vaccines that induce protective neutralizing antibodies against a broader spectrum of coronaviruses. This push will require a better understanding of the glycan shields of coronaviruses that help limit the access of neutralizing antibodies to epitopes on the spike glycoproteins that decorate the surface of the viruses. In fact, approximately one third of the mass of the spike glycoproteins consists of N-linked glycans and variations in the glycan shield between coronaviruses help define conserved epitopes that are recognized by antibodies able to neutralize multiple coronaviruses (broadly neutralizing antibodies). Such antibodies should be induced by pan-coronavirus vaccines. In this study, we produced 10 soluble stabilized recombinant sarbecovirus glycoproteins and analyzed their glycan shields by mass spectrometry. The majority of N-linked glycan attachment sites are shared by all sarbecoviruses analyzed in the study, but there are significant differences in the processing state at glycan sites on and around the receptor binding domain. Additionally, certain sites are highly conserved with respect to both position, and glycan processing, highlighting the key role that N-linked glycans perform in stabilizing the structure of the sarbecovirus spike glycoprotein. Overall, our studies reveal important similarities and differences in the glycosylation of sarbecovirus Envelope glycoproteins and provide valuable data for pan-sarbecovirus vaccine design

Poster 2

Towards real-time glycopeptide identification on the timsTOF pro-PaSER platform

Authors:
Gad Armony[1], Sven Brehmer[2], Lennard Pfennig[2], Sebastian Wehner[2], Fokje Zijlstra[1], Tharan Srikumar[3], Gary Kruppa[4], Dirk Lefeber[1,5], Alain van Gool[1], Hans Wessels[1]
1 Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, 6525 GA Nijmegen, The Netherlands
2 Bruker Daltonics GmbH & Co. KG, 28359 Bremen, Germany.
3 Bruker Ltd, L9T 6P4 Milton, Canada.
4 Bruker S.R.O., 61900 District Brno-City, Czech Republic.
5 Department of Neurology, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, 6525 GA, Nijmegen, The Netherlands.

Protein glycosylation greatly affects protein function and may serve as useful biomarkers in biomedical applications. Holistic glycoproteomics in blood can provide site-specific data for glycosylation of up to hundreds of proteins in a single measurement and therefore may be applied to functional diagnoses of human diseases. We developed software modules for the PaSER (Parallel Search Engine in Real-time) computational platform that perform (semi) real-time data processing, and analysis. The modules exploits the common N-glycan core structure to determine the mass of the peptide moiety part of the glycopeptide. The peptide moiety is then identified by the database search engine in PaSER (ProLuCID) using the determined peptide moiety mass. We optimized the acquisition and analysis parameters in a data-driven manner to identify plasma glycopeptides using relevant control plasma samples. We show that the glycopeptide identification is faster than data acquisition so it can indeed be performed in real-time. To benchmark the identification performance, we compare it to MSFragger-glyco and show that the vast majority of the identifications (92%) overlap between the two tools. As the identification is done during data acquisition, the results are available for subsequent downstream analysis right after the end of the LC-MS/MS measurement. This enables building automated pipelines for glycopeptide diagnostics and glycoproteomics in clinical environments.

Poster 3

Mechanism of mucin cross-feeding between Ruminococcus gnavus strains.

Wouter van Bakel[1], Dimitrios Latousakis[1], Emmanuelle Crost[1], Haiyang Wu[1], Ana Martinez-Gascuena[1], Nathalie Juge[1]

[1] Quadram Institute Bioscience

Ruminococcus gnavus is a prevalent gut symbiont in both adults and infants that can utilise human milk oligosaccharides (HMOs) and mucin-derived glycans. The glycan foraging ability of *R. gnavus* is strain specific. Here we used RNAseq to identify the range of glycoside hydrolases (GHs) involved in mucin glycan utilisation by *R. gnavus* ATCC 29149. We confirmed that the Nan cluster dedicated to sialic acid metabolism was the most induced cluster followed by three fucosidase genes. We identified a 10-gene operon containing a highly upregulated GH98 endo-β-1,4-galactosidase, a GH95 fucosidase and GH73 N-acetylglucosaminidase. We showed that the recombinant *Rg*GH98 was highly specific to the type II Blood Group A (BgA) epitope, releasing BgA trisaccharide (BgAtri). This specificity may confer regio-selectivity to *R. gnavus* strains along the length of the colon and contribute to the early adaptation of *R. gnavus* to the infant gut since HMO profile is determined by both secretor and Lewis status. In addition, we showed using MALDI-TOF MS and HPAEC-PAD that the released BgAtri could be utilised by *R. gnavus* E1, a strain that does not encode a GH98 and is otherwise unable to grow on mucin as the sole carbon source. Additional MS analyses showed that *R. gnavus* E1 was also able to utilise the underlying mucin glycans after release of BgA. This cross-feeding interaction could play an important role for the survival of *R. gnavus* strains that cannot directly utilise mucin glycans in the GI tract.

Poster 4

The Impact of Crowding on Membrane-Associated Specific Binding Between High-Precision Glycomacromolecules and Lectins

Luca-Cesare Blawitzki[1], Lorand Bonda[1], Stephan Schmidt[1], Cornelia Monzel[2], Laura Hartmann[1]

[1]Heinrich-Heine-University Duesseldorf, Institute of Organic and Macromolecular Chemistry, Universitaetsstrasse 1, 40225 Duesseldorf, Germany; ***laura.hartmann@hhu.de***

[2] Heinrich-Heine-University Duesseldorf, Institute of Experimental Medical Physics, Universitaetsstrasse 1, 40225 Duesseldorf, Germany

Cellular membranes are complex entities that are densely functionalized with a heterogenous composition of lipids and proteins. The majority of these membrane constituents are heavily glycosylated and create a dense layer of carbohydrates covering the cell surface, which is referred to as the glycocalyx. This layer of glycans that serves as a protecting barrier to the cell is also known to be actively involved in many recognition events, such as intercellular communication or pathogen invasion. However, the details of these cellular processes that are mediated by biophysical mechanisms are yet to be fully understood.

The diversity and heterogeneity of the glycocalyx, combined with the dynamic adaptation of the cell membrane to external stimuli, makes it almost impossible to assign individual biological events to single structural components. Engineering of giant unilamellar vesicles (GUVs) with artificial constituents is one strategy to mimic cellular membranes in a highly controlled fashion, thereby enabling a systematic examination of component properties in particular interaction events.

Here, we present our recent work on the synthesis of defined mimetics of crowded glycocalyces. Inspired by natural glycocalyx constituents, we applied solid-phase synthesis in combination with controlled radical polymerization to synthesize multivalent glycolipid-analogues of different lengths. Each structure thereby comprises a defined glyco-head group, a cholesteryl-moiety for membrane tethering and a fluorescent marker for visualization. Through incorporation of these structures in distinct combinations into GUVs, we can then create precise mimetics of crowded glycocalyces with adjustable carbohydrate sequence and density. Combining optical microscopy and fluorescence microscopy allows us to study the interaction of these proto-glycocalyces with well-studied lectins as model systems for glycan-mediated host-pathogen interactions in a densely crowded environment

Poster 5

MICROBIAL-GLYCOENGINEERING APPROACH TO IMMUNOTHERAPY

F. Balzarini, S. Bruijns, A. Marseglia, A. Silipo, R. Marchetti, F. Chiodo and Y. Van Kooyk

Department of Molecular Cell Biology and Immunology, Amsterdam University Medical Center, Amsterdam Institute for Infection and Immunity, Vrije Universiteit Amsterdam, Amsterdam Netherlands. Centro Nazionale delle Ricerche (CNR), Universita`degli studi Federico II, Napoli, Italy. Finlay Institute, Havana, Cuba. Marie Skłodowska-Curie Actions, International Training Network Glytunes, European Union.

The dialogue between the innate and the adaptive branches of the immune system is critical for protection against infections, as well tumor, autoimmune, allergic and inflammatory diseases.

In past decade, our understanding of the activation of the innate immune system through pattern recognition receptor (PRR) improved a lot and it helped to better understand the mechanisms to drive immune responses and to increase and adjuvate the outcome of vaccination and immunotherapy. PRR, like C-type Lectin (CLR), I-type Lectin (ITL, also called Siglecs), Toll-like Receptor (TLR) and Nod-like receptors (NLR), recognize molecular motives present in the environment and induce antigen presentation, signal transduction and production of effector molecules in innate immune cells.

Most of the studies have been done by triggering a single PRR with a specific synthetic (or isolated) molecular pattern but pathological conditions are much more complex and there are always multiple activations of different PRRs from different molecular patterns or a single complex molecule that is able to trigger different PRRs.

Biomarker modification through glycosylation emerged to have a strong immune modulatory effect by inducing altered targeting via CLR and Siglecs. Using glycosylated microbial molecules and particles as PRRL, like capsular polysaccharide (CPS) and outer-membrane vesicles (OMV) isolated from bacteria, we will improve vaccine formulations, with the ultimate aim to test them in tumor mouse models.

We included these PRRL in different engineered formulations to create a Microbial Adjuvanted Glycosylated Molecules (MAGMs) by absorbing them on Aluminium Hydroxide (Alum) or by encapsulation in PLGA nanoparticles. MAGM help to induce a multiple PRR targeting that were tested in human monocyte to understand the series of immune responses. As results, the outcome generated after MAGM stimulation is dependent by the activities of CTL and Siglecs which enhance APC targeting and avoid possible side effects of the vaccination strategy.

Poster 6

Effect of fungal chitin as a curative or preventive treatment on *C. glabrata* elimination and modulation of intestinal inflammation in a dextran sulphate sodium (DSS)-induced colitis mouse model.

Rogatien Charlet[1.2.3], Karine Lecointe[1.2.3], Clovis Bortolus[1.2.3], Boualem Sendid[1.2.3] & Samir Jawhara[1.2.3]

[1] CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale Et Fonctionnelle, INSERM U1285, 59000, Lille, France.

[2] University of Lille, 1 place Verdun, 59000, Lille, France.

[3] CHU Lille, Service de Parasitologie Mycologie, Pôle de Biologie Pathologie Génétique, 59000, Lille, France.

In a healthy individual, the intestinal microbiota is stable over time and represents a natural barrier to pathogen proliferation. However, a "dysbiosis" of the microbiota can occur during certain pathological conditions or drug treatments resulting in an invasion and multiplication of opportunistic pathogens. Among them is *Candida glabrata* (*C. glabrata*), an opportunistic pathogenic yeast, capable of colonizing the digestive tract under certain pathophysiological conditions favoring its translocation through the digestive epithelial barrier and its subsequent dissemination. However, it is essential to underline that the interactions established between *C. glabrata* and the host are mainly through its wall. In this context, our laboratory focuses on the components of this wall and more precisely on the role and properties held by fungal chitin, a natural β-(1,4)-N-acetyl-D-glucosamine polysaccharide. Our previous data show that this biopolymer holds protective properties against DSS-induced colitis in mice. Indeed, oral administration of this chitin attenuated the deleterious effects of colitis while reducing the proliferation of pathobionts such as *Escherichia coli*, *Enterococcus faecalis* or *C. glabrata*.

Nevertheless, despite a better understanding of the properties of this polysaccharide, the mechanisms of action and the interactions involving immunity are still poorly defined. Thus, in this same mouse model, we confirmed that a curative treatment with chitin has protective properties against colitis. Moreover, we note that mice under treatment show a decrease in inflammatory parameters correlated with a reduction in fungal and bacterial loads. These treated mice show a modulation of the expression of PPARγ _or TLR9, involved in the anti-inflammatory response, while decreasing the expression of key inflammatory mediators of innate immunity. In parallel, we performed a preventive treatment with chitin in order to better understand its role in the elimination of *C. glabrata* as well as in the mechanisms related to the immune system. Our data show that this treatment allows the development of antibodies directed against chitin while allowing mice under inflammation to better resist *Candida* colonization.

Key words : *C. glabrata*, fungal chitin, Immunity

Poster 7

Glycan engineering of cardiac progenitor cell-derived extracellular vesicles.

Charlotte Clarisse[1], Claire Nouguier[2], Valérie Bellamy[2], Manon Desgres[2], Thomas Viel[3], Claire Provost[4], Bertrand Tavitian[3,5], Anne Harduin-Lepers[1], Robert Sackstein[6], Philippe Menasché[2,7], Sophie Groux-Degroote[1]

[1]Univ-lille, UMR 8576 Unité de Glycobiologie Structurale et Fonctionnelle (UGSF), F-59000 Lille FR

[2]Univ-Paris Inserm U970, Paris Centre de Recherche Cardiovasculaire (PARCC) Team 6, Regenerative Therapies for cardiac and vascular diseases, F-75015 Paris FR

[3] Univ-Paris Inserm U970, Paris Centre de Recherche Cardiovasculaire (PARCC) Team 2, *In vivo* Imaging Research Laboratory, F-75015 Paris FR

[4]Insitut Curie, U1288 Inserm, Laboratory of Translational Imaging in Oncology, F- Orsay FR

[5] Service de radiologie, AP-HP, Hôpital européen Georges Pompidou, F-75015 Paris.

[6] Warrior Therapeutics, Cambridge, MA, USA

[7] Department of Cardiovascular Surgery, Hôpital Européen Georges Pompidou, 20, rue Leblanc, F-75015 Paris, France

There is now evidence that in heart failure, the primary mechanism of action of stem cells is not the physical replacement of the damaged cardiomyocytes but rather a paracrine harnessing of endogenous repair pathways by cell-secreted biomolecules which include soluble factors and extracellular vesicles (EV). Our major research hypothesis is that EV present in the secretome of cardiovascular progenitor cells (CPC) can be glycan-engineered to improve their cardiac targeting, which should promote a better recovery of function in a rodent model of myocardial infarction. A major hallmark of heart failure is a myocardial inflammation also involving the vascular endothelium. This inflamed endothelium expresses an adhesion molecule, E-selectin, the presence of which has been documented in blood and in myocardial biopsies of heart failure patients. Our objective is to increase the cardiac homing of intravenously injected EV by forcing them to express E-selectin-specific ligands through engineering of their glycan signature.

The canonical binding determinant for E-selectin is the tetrasaccharide known as “sialyl Lewis X” (sLeX; NeuAcα(2,3)Galβ(1,4)[Fucα(1,3)]GlcNAc-R). The CPC are differentiated from induced pluripotent stem cells (iPSC). Following a final expansion phase under serum-free conditions, the EV-enriched secretome is collected through a series centrifugations and qualified for the number of particles, EV surface markers and bioactivity using a cell survival potency assay.

The glycosignature of CPC-derived EV was studied using a combination of lectins (MAL-1, MAL-2, AAL) and antibodies (anti-sLe^X CD15s, HECA-452), using western/lectin blotting and flow cytometry. Our results suggest that CPC-derived EV do not express sLe^X at their surface in the control conditions. Consequently, aiming at increasing sLe^X expression, we performed exofucosylation experiments, using α1,3-fucosyltransferases FT6 or FT7, and exosialylation (using α2,3-sialyltransferase ST3GAL4 or ST4GAL4). Our flow cytometry data suggest that the expression of sLe^x on CPC- derived EV is increased after exofucosylation, and potentially even more after exosialylation followed by exofucosylation, which could affect EV biodistribution in rodent model of myocardial infarction.

Poster 8

REDUCTION OF SURFACE SIALIC ACID AS A POTENTIAL TOOL AGAINST CANCER

Magali Coccimiglio[1,2,3], Kelly Boelaars[1,2,3], Thomas Boltje[5], Fabrizio Chiodo[1,4], Yvette van Kooyk[1,2,3]

[1]Dept. of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, the Netherlands
[2]Amsterdam Infection & Immunity Institute, Amsterdam, the Netherlands
[3]Cancer Center Amsterdam, the Netherlands
[4]Institute of Biomolecular Chemistry, National research Council (CNR-ICB), Naples, Italy
[5]Institute for Molecules and Materials, Radboud University, Nijmegen, the Netherlands
m.b.coccimiglio@amsterdamumc.nl

During tumorigenesis, changes in glycosylation occur, leading to the aberrant expression of different surface glycans on tumor cells. Particularly, hypersialylation has been identified as a major hallmark in many cancer types. Sialic acid can be recognized by Sialic acid binding immunoglobulin type lectins (Siglecs) on immune cells. As most Siglecs have immune inhibitory potential and are expressed on a variety of immune cells, sialic acids are considered immune checkpoints that control the suppressive state of the tumor immune microenvironment, and could determine immunotherapy effectiveness.

To control sialylation in tumor development, we used a global sialyltransferases inhibitor to reduce surface sialic acids on different tumor cell lines. We characterized the expression of sialic acid ligands for different Siglecs upon treatment. Current experiments are focussed on whether this inhibition alters the tumor microenvironment in 3D co-cultures and on its in vivo application to increase anti-tumor immune responses, making the sialylation pathway a potential target for combinatorial therapies.

Poster 9

Integrating metabolomics and 3D engineered heart tissue for disease modelling and therapy-screening of metabolic cardiomyopathies

Authors (presenting author underlined:
[1,2]Conte Federica, [1]Noga Marek, [2]Cofino-Fabres Carla, [2]Danique Snippert, [3]van Rooij Arno, [2]Passier Robert, [1,3]Lefeber Dirk.

Affiliations:
(1) Department of Neurology, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, NL
(2) Department of Applied Stem Cell Technologies, Faculty of Science and Technology, University of Twente, Enschede, NL
(3) Translational Metabolic Laboratory, Radboud University Medical Center, Nijmegen, NL

Abstract (max 400 words):

Congenital heart defects can result from a wide range of genetic mutations that often effect the contractile or metabolic apparatus of cardiac cells. Traditionally, cardiomyopathies have been modeled in vivo using genetically modified animals that have significantly contributed to the mechanistic understanding of disease, but often fail to fully replicate human pathology. Recent breakthroughs in tissue engineering allowed the generation of patient-derived 3-dimensional engineered heart tissue (3D-EHT, a.k.a. 'heart-on-chip') to study cardiac physiology *in vitro*. However, cardiac metabolism in these models remains unexplored, and with it the possibility to use 3D-EHTs to study metabolic cardiomyopathies.

Metabolic cardiomyopathies result from a wide range of genetic mutations that affect the contractile machinery and metabolism of cardiac cells. Traditionally, cardiomyopathies have been modeled i*n vivo* using genetically modified animals which significantly contributed to the mechanistic understanding of these defects, but fail to fully recapitulate human pathophysiology. Recent breakthroughs in the field of tissue engineering allowed the generation of patient-derived 3D engineered heart tissue (3D-EHT, a.k.a. ' heart-on-chip') to study human cardiac defects *in vitro*. However, cardiac metabolism in these models remains unexplored, and with it the possibility to use 3D-EHTs to study metabolic cardiomyopathies.

In our project, we aim to establish a platform that integrates functional patient-derived 3D-EHTs with metabolomics, to characterize the metabolic mechanisms underlying the cardiac defects in metabolic cardiomyopathies, while identifying new potential therapeutic targets.
To establish this platform, we chose phosphoglucomutase (PGM1) deficiency as disease model. First, we generated induce pluripotent stem cells (iPSCs) from patients, and developed a methodology to study the carbohydrate metabolism via LC-Mass spectrometry at steady-state and dynamically flux analysis using stable isotope tracing. Second, we differentiated these patient-derived iPSCs to generate 3D-EHTs. Third, we adapted our metabolomics pipeline to suit 3D-EHT models, enabling the extraction of polar metabolites for LC-MS after functional assays (e.g. force of contraction quantification).
Here, we showcase our analytical platform, along with the data on carbohydrate metabolism in control and patient-derived iPSCs (both at steady-state and dynamic flux analysis) and 3D-EHTs (steady-state analysis).
In the next phase, we aim to further extend this platform and exploit it to (a) further deepen the metabolic characterization of PGM1-deficient cardiac tissue, and (b) test potential therapeutic treatments both on a functional and on a metabolic level. This phase will also provide the proof-of-principle for broadening the application of our platform to personalize pre-clinical therapy-screening for other congenital and acquired cardiomyopathies.

Poster 10

Rational Design of α-1,4-Galactosyltransferase Inhibitors as Substrate Reduction Therapy for the Treatment of Fabry Disease.

N. de Koster[a], K. Kok[a], J. Hoogervorst[a], R. E. Katzy[a], L. T. Lelieveld[a], M. J. Ferraz[a], H. S. Overkleeft[b], J. M. F. G. Aerts[a], M. E. Artola[a]
Departments of Medical Biochemistry[a] and Bio-organic Synthesis[b], Leiden Institute of Chemistry (LIC), Leiden University, 2300 RA Leiden, The Netherlands.

Background: Fabry disease (FD) is a lysosomal storage disorder, characterized by the deficiency of the enzyme α-galactosidase A (α-GalA) and the consequent toxic build-up of glycolipids including Gb3 and lyso-Gb3. The lack of efficacy of existing therapies which focus on restoring α-GalA activity emphasizes the need for novel approaches to treat FD.[1] Alternatively, substrate reduction therapy targeting the enzyme responsible for Gb3 biosynthesis, α-1,4-galactosyltransferase (A4GALT), could prove a viable strategy. For this purpose, effective inhibitors of A4GALT are required, which so far have not yet been identified.
Methods: We have based our rational design of A4GALT inhibitors on the known substrate glycolipid lactosylceramide (LacCer). We have generated a library of compounds functionalized with a range of reactive moieties as a means to bind the A4GALT active site and impair galactosylation. Their inhibitory potency can be assessed by either an *in vitro* HPLC-UV method or via *in situ* $^{13}C_5$-sphingosine ($^{13}C_5$-Sph) isotope feeding assay followed by UPLC-MS/MS analysis.[2] Two adamantyl-substituted LacCer analogues previously described to affect glycosphingolipid metabolism in cells[3], AdaGalCer and AdaGlcCer, have been used for assay optimization as positive controls.
Results: Currently, a family of 13 LacCer mimetics have been synthesized and evaluated. $^{13}C_5$-Sph feeding experiments in human fibroblasts revealed several LacCer analogues able to lower the glycosphingolipid levels in the Gb3 pathway, including three compounds that reduce Gb3 levels with estimated IC_{50} values ranging from 43 to 86 μM and are selective over related glycosidases and glycosyltransferases.
Conclusion: We provide the means to assess glycosylation activity of A4GALT in an *in vitro* and *in situ* cell setting. Several micromolar inhibitors of Gb3 biosynthesis have been identified, which will aid the rational design in future optimization steps of A4GALT inhibitors for the treatment of FD.

Support: We thank The Netherlands Organization for Scientific Research (NOW-CW, ChemThem grant) and the European Research Council (ERC-2020-SyG-951231 "Carbocentre").
References: [1]Kok *et al. Biomolecules* **2021**, 11(2), 271. [2]Mirzaian, M. *et al. Clin. Chim. Acta* **2017**, 466, 178–184. [3]Kamani *et al. J. Biol.Chem.* **2011**, 286(24), 21413-21426.
Keywords: galactosyltransferase, glycosphingolipid, ^{13}C-sphingosine feeding assay, mass spectrometry, Fabry disease.
inflammation. We provide evidence for a low-grade chronic intestinal inflammation after pelvic radiotherapy, that may drive the progression of disease in the irradiated colorectal mucosa.

Poster 11

A method platform to make intact-glycoproteomics more accessible

Marc D. Driessena Ulla I.M. Gerling-Driessenb, Kai Stuehlera,c

a Heinrich-Heine-University Duesseldorf, Proteome Research, Institute of Molecular Medicine I, Duesseldorf, Germany
b Heinrich-Heine-University Duesseldorf, Institute of Organic and Macromolecular Chemistry, Duesseldorf, Germany
c Heinrich-Heine-University Duesseldorf, Molecular Proteomics Laboratory, BMFZ, Duesseldorf, Germany

Mapping changes in the glycoproteome remains challenging. Yet a wealth of biological information can be gained, that is inaccessible via standard/quantitative proteomics. Currently, incorporating glycoproteomic data in biological evaluations is still challenging due to complexity and inaccessibility, especially when analyzing intact glycopeptides. We currently develop a modular method-platform that can be used to facilitate experiments from a first 'fingerprinting' to a detailed analysis of the glycosylation status of complex biological systems. We aim to establish a set of 'pick and choose' standard protocols for the crucial steps of sample preparation.
One aspect of this platform will be the application of chemo-proteomic solutions via the use of modular, bioorthogonal probes. We plan to combine multi-functional, cleavable probes and stable-isotope labeling to facilitate enrichment and quantitative analysis through the same probe. Basis for this is metabolic engineering of glycans to incorporate bioorthogonal handles. A first-generation library contains probes with isotope labelled core and various cleavable linkers combined with two or more functional units for combined workflows (e.g. imaging, enrichment, photo-crosslinking, etc.)
Our platform relies on versatility, robustness, ease of use and time efficiency. Currently the whole workflow takes approx. 6.5 hours to prepare 6-12 samples for analysis. A fast first analysis is capable of producing a glycoproteomic fingerprint and captures even facile differences in N-glycosylation. Using models for either cancer or congenital disorders of glycosylation (CDGs), we mapped several hundred intact glycopeptides on proteins spanning from ER-resident to cell-surface proteins. Combined with current advances and availability of relevant data analysis tools, we believe that this method platform can help to lower the hindrance to include glycoproteomic analysis in biological projects, thereby giving access to this often-unexplored information.

Poster 12

Nutrient regulation of gene expression by O-GlcNAcylation of basal transcription machinery

Louis Fréville[1#], Carlos López Gómez[2#], Quentin Lemaire[1], Partha S. Banerjee[4], Guanghui Han[4], Junfeng Ma[4], Gerald W. Hart[4], Audrey Vincent[3], Tony Lefebvre[1] and Stéphan Hardivillé[1*]

[1]Unité de Glycobiologies Structurale et Fonctionelle, UMR 8576 CNRS - Université de Lille, Faculté des Sciences et Technologies de Lille, Villeneuve d'Ascq, France
[2]IBIMA - UGC Endocrinology, Diabetes, Obesity and Nutrition - Hospital Regional de Malaga, Málaga, Spain
[3]CANTHER, UMR-S 1172 Inserm – Université de Lille, Lille, France
[4]Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

ABSTRACT

Complex molecular mechanisms ensure cellular homeostasis between nutrient sensing and energy metabolism. Variation in nutrient availability is reflected by flux through the hexosamine biosynthetic pathway and the concentration of its end-product, UDP-GlcNAc. This nucleotide sugar serves as the donor substrate for *O*-GlcNAc transferase (OGT) catalyzed *O*-GlcNAcylation of myriad nuclear, cytoplasmic and mitochondrial proteins. OGT enzymatic activity is not only highly responsive to UDP-GlcNAc across a very broad range of concentrations but also its substrate specificity is dependent upon the concentration of the nucleotide sugar. OGT acts then as a "control center" that integrates metabolic flux to send signals to effectors *via O*-GlcNAcylation. Many examples of regulation of cellular signaling pathways by *O*-GlcNAcylation have been reported, but the *O*-GlcNAc nutrient regulation of basal transcription mechanisms remains poorly understood.

Gene expression is performed by three RNA polymerases that require the assembly of specific molecular complexes at gene promoters to initiate transcription. Among them, the TATA-Box Binding Protein (TBP) was viewed as a scaffold with a passive role in gene expression regulation. Our recent data showed that TBP is targeted by the nutrient-sensitive *O*-GlcNAcylation, regulating its interaction with BTAF1, hence the formation of the B-TFIID complex. Dysregulation of TBP *O*-GlcNAcylation affects basal transcription processes, altering the expression of genes involved in lipid metabolism. Sustained hyperglycemia as occurs in patients with diabetes is associated with macro- and micro-vascular complications and a significant risk of different forms of cancer including colorectal cancer. An increase of glucose-dependent *O*-GlcNAcylation of TBP alters the expression of genes involved in the early onset of colorectal tumorigenesis. Our data bring the first mechanistic insight on how to hyperglycemia-induced TBP *O*-GlcNAcylation plays an essential role in linking the etiology of colorectal cancer and diabetes.

Poster 13

Importance of *C*-mannosylation for function of semaphorins

Alina Friedrich[1], Karsten Cirksena[1], Falk F. R. Buettner[1]

[1] Institute for Clinical Biochemistry, Hannover Medical School, Hannover, Germany

C-Mannosylation describes the covalent attachment of a mannose to tryptophan residues within the consensus sequence WxxW/C of target proteins via a C-C bond. This reaction is catalyzed in the lumen of the ER by a C-mannosyltransferase which has been initially identified in *C. elegans* as DPY19[1]. In mammals four homologues of DPY19 designated as DPY19L1 to L4 are present of which DPY19L1 and DPY19L3 have been proven to be active C-mannosyltransferases[2]. Target proteins for *C*-mannosylation comprise the thrombospondin type one repeat (TSR) domain-containing proteins, e.g. the *C. elegans* protein MIG-21[1] and its mammalian homologue SEMA5[3]. Both proteins have been shown to be involved in neuronal migration but the importance of C-mannosylation for their function is not known.[4,5].

SEMA5A and SEMA5B contain multiple TSRs and in order to map and quantitatively determine the degree of C-mannosylation, fragments comprising different TSRs of these proteins were recombinantly expressed in wild-type, DPY19L1- and DPY19L3-deficient CHO cells and analyzed by mass spectrometry. This analysis showed the C-mannosylation of several tryptophan residues of different TSRs of SEMA5A and SEMA5B in CHO wild-type cells. The recombinant expression in the DPY19 L1 or L3 KO cell line results in partial C-mannosylation of the consensus sequence. We further deleted the C-mannosyltransferases DPY19L1 and DPY19L3 in human induced pluripotent stem cells (hiPSC) by CRISPR-Cas9 which were then differentiated into neurons endogenously expressing SEMA5A. Initial experiments suggest that SEMA5A is not localized at its target location at the plasma membrane in DPY19L1-deficient hiPSC-derived neurons.

[1] Büttner et al. (2013), C. elegans DPY-19 is a C-mannosyltransferase glycosylating thrombospondin repeats, Mol Cell 50, 295-302
[2] Shcherbakova et al (2017), Distinct C- mannosylation of netrin receptor thrombospondin type 1 repeats by mammalian DPY19L1 and DPY19L3, PNAS 114, 2574-2579
[3] John et al (2021), Yeast- and antibody-based tools for studying tryptophan C-mannosylation, Nature Chemical Biology 17, 428-437
[4] Middelkoop et al (2012), The thrombospondin repeat containing protein MIG-21 controls a left-right asymmetric Wnt signaling response in migrating C. elegans neuroblasts, Developmental Biology 361, 338-348
[5] Adams et al. (1996), A novel class of murine semaphorins with homology to thrombospondin is differentially expressed during early embryogenesis Mechanisms of Development 57, 33-45

.

Poster 14

Immunoglobulin G N-glycosylation changes in coronary disease

Barbara Radovani[1], Gordan Lauc[2,3], Ivan Gudelj[1,2*]

[1] Department of Biotechnology, University of Rijeka, Rijeka, Croatia
[2] Genos Glycoscience Research Laboratory, Borongajska cesta 83h, Zagreb, Croatia
[3] Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, Zagreb, Croatia

Cardiovascular diseases are the leading cause of mortality and morbidity in the world and knowing risk factors and molecular mechanisms that are crucial for their development has greatly contributed to their prevention and early treatment. Coronary artery disease (CAD) is the most prevalent cardiovascular disease and glycosylation changes have already been associated with their development. However, alterations of immunoglobulin G (IgG) N-glycosylation have not been studied, even though IgG N-glycans are known to affect its effector functions which may lead to modulation of the inflammatory response in CAD. Therefore, in this study we isolated IgG using affinity chromatography from individuals with coronary atherosclerosis (CAD+) and individuals with clean coronaries (CAD-) who are suspected of CAD developing. Purified IgGs were denatured and enzymatically deglycosylated. The released and fluorescently labelled N-glycans were analyzed by ultra-high performance liquid chromatography with hydrophilic interaction analysis. Obtained chromatograms give an insight into IgG glycome composition in CAD, and the biomarker potential of IgG N-glycans for prediction and early diagnosis of CAD.

Poster 15

Title: Discovery of a new family of O-GlcNAc Transferase inhibitors by DNA encoded library screening

Cyril Balsollier

O-GlcNAcylation is a post-translational modification of attaching a β-N-Acetylglucosamine to serine and threonine residue of proteins. This post-translational modification is competing with phosphorylation and therefore has an important role in several biological systems Driven by only two enzymes O-GlcNAc Transferase and O-GlcNAcase. Dysregulation of the O-GlcNAcylation system has been reported in degenerative diseases like cancer, diabetes, and Alzheimer's which makes OGT and OGA potential therapeutic targets. While the field of OGA inhibitors has reached the stage of clinical trials, OGT inhibitors are still needed. Actual OGT inhibitors like OSMI-4 are potent but are lacking pharmacochemical properties. The Discovery of new OGT inhibitors is impaired by the stability of the enzyme and the absence of a reliable High-Throughput-Screening binding assay. DNA encoded libraries are chemical libraries where chemicals are identified by a unique DNA strain. This technique is gaining interest in the medicinal chemistry field since through a simple binding assay it is possible to fish out potential binders out of millions. In this work, we are reporting the discovery of a new family of OGT inhibitors using DNA encoded library screening. This new family of inhibitors is also showing a new binding mode that we are trying to elucidate through computational chemistry.

Poster 16

Glycocalix[4]arene Derivatives for the Functionalization of Ultrasmall Gold Nanoparticles

Alisa Kayser[1], Kai Klein[2], Daria Babushkina[1], Gisele Mouafo Kenne[1], Matthias Epple[2], Laura Hartmann[1]

[1]Heinrich-Heine-Universität, Universitätsstraße 1, 40225 Düsseldorf, Germany, laura.hartmann@hhu.de
[2]Universität Duisburg-Essen, Universitätsstr. 7, D-45141 Essen, Germany

Gold nanoparticles as well as calixarenes are well-known platforms to create multivalent carbohydrate ligands to improve binding avidity and selectivity towards carbohydrate-recognizing receptors. Here we present a combination of both scaffolds giving higher control yet also flexibility in the synthesis and design of carbohydrate-calixarene-gold nanoparticle conjugates.
First we adapted a synthetic protocol for a calix[4]arene building block carrying a single carboxy-group on the lower rim. After addition onto the solid support using standard peptide coupling conditions, the upper rim functionalities are reduced to give four amine groups. These can then again be used for elongation of functionalization by previously established solid phase polymer synthesis. Applying this method, two functionalized calix[4]arene derivates were derived, either carrying four carbohydrate moieties at the upper rim and one alkyne moiety at the lower rim or vice versa.
Next, using copper mediated click chemistry the glycocalix[4]arene can be conjugated onto azido-functionalized ultrasmall gold nanoparticles via their alkyne functionalities. We hypothesized that the two calix[4]arene derivatives should result in different numbers of carbohydrate ligands on the gold nanoparticle surface. First studies to examine carbohydrate concentration on the nanoparticles by sulfuric acid-phenol method confirm this with the ability to now vary the number of carbohydrates from 18 to 152 per nanoparticle. Finally, the glycocalix[4]arene functionalized ultrasmall gold nanoparticle are successfully evaluated in biological assays as inhibitors of bacterial adhesion correlating the multivalent display of Mannose units with their ability to efficiently block *E.Coli* adhesion.

Poster 17

Deciphering the impact of Ruminococcus gnavus cell surface glycosylation at the mucosal interface

Laplanche Victor[1], Armiento Samantha[2], Šuligoj Tanja[1], Crost Emmanuelle Helene[1], De Castro Cristina[3] and Juge Nathalie[1].

[1]Gut Microbes and Health Programme, Quadram Institute Bioscience, Gut Microbes and Health Institute Strategic Programme, Norwich NR4 7UQ, UK.
[2] Department of Chemical Sciences, Via Cintia 4, 80126 Napoli, Italy.
[3] Department of Agricultural Sciences, University of Naples, 80055 Portici, Italy.

The gut microbiota plays a major role in human health and an alteration in gut microbiota structure and function has been implicated in several diseases. *R. gnavus* is an important member of the 'normal' gut microbiota and over-represented in inflammatory bowel disease. There is therefore great interest in understanding the mechanisms underpinning its interaction and communication with the host. Here we aim to gain mechanistic insights into *R. gnavus* immunomodulatory properties by studying wo aspects of the subject: first, this project intend to underpin the cell surface glycosylation composition of the bacteria, and then correlate it to the interaction with host immunity modulation in a strain-dependent manner. To address this first point, flow cytometry was used to screen cell surface glycosylation of different *R. gnavus* strains including *R. gnavus* E1, ATCC 29149, ATCC 35193) using a range of 9 lectins. The results showed differences in the lectin binding profile between the different strains tested, suggesting strain-specific differences in carbohydrate epitopes on the cell surface. These differences were supported by bioinformatic analyses revealing differences in the biosynthetic cluster for glucorhamnan, a major capsular polysaccharide characterised in *R. gnavus* ATCC 29149 with inflammatory properties *in vitro*. To validate these findings, the polysaccharides present on E1 and ATCC 35913 strains cell surface were structurally characterised using NMR and mass spectrometry. The analysis revealed a backbone composed of four (α2,1) and (α3,1)linked rhamnose and sidechains composed of one (β1,3)linked glucose, therefore different from the previously reported structure of ATCC 29149, validating our previous data. In addition, for one given strain, we showed that the carbohydrate used to grow the bacteria (glucose, fucose, galactose, N-acetylglucosamine, 3'-fucosyllactose, lactose, 2'-fucosyllactose, sucrose or 3'-sialyllactose) influenced the lectin binding profile, suggesting the bacteria cell surface carbohydrate composition was dependent on the carbohydrate source. These differences in cell wall glycosylation may influence the immune response and this is currently being investigated using immunoassays with immune cells (murine BMDC) and intestinal epithelial cells (T84 and LS174T cell lines), which showed us evidence that the strains and their associated isolated glucorhamnan enhanced the activation of the host immunity in a strain specific manner.

Poster 18

Using mucormycosis extracellular polysaccharide as a target: a lead to a new biomarker?

Karine Lecointe[1], Pauline Coulon[1], Léna Noailles[1], Marjorie Cornu[1], Boualem Sendid[1]

[1]Univ. Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, Lille, France; Univ. Lille, Inserm U1285, CHU Lille, Laboratoire de Parasitologie-Mycologie, Lille, France.

Objectives. Extracellular polysaccharides (EPSs) from fungi (filamentous or yeast, Ascomycota and Basidiomycota mainly explored) have been shown to exhibit significant biological activities, including immunomodulation, antitumor, antioxidant, anticancer and antiviral activities. Mucormycosis (MM), an emerging angio-invasive fungal infection, is associated with high morbidity and mortality. Data regarding biochemical characteristics and biological properties of EPSs from Mucorales are scarce. The objectives were to analyze the sugar content and linkage pattern of EPSs from Mucorales and the presence of antibodies against EPS in patients'sera.

Methods. Two strains of *Lichtheimia corymbifera* (one reference strain IHEM 21658; one clinical isolate) and one clinical isolate of *Rhizopus arrhizus* were used. After 7 days of incubation in yeast nitrogen base media supplemented with glucose 0,5%, the culture fluid was precipitated with 4 volumes of ethanol at 4°C before deproteinization using the Sevag method, to isolate EPSs. Monosaccharide composition of EPSs was determined by gas-chromatography using flame - ionisation detector. Serum samples from 10 healthy subjects, 15 patients with invasive aspergillosis, 10 patients with candidemia and 51 sera from 12 patients with mucormycosis (CHU Lille, France) were collected. The presence of antibodies directed against EPSs of Mucorales was assessed by a direct enzyme-linked immunosorbent assay method.

Results. Fucose, glucose, mannose, galactose, N-acetylglucosamine (GlcNAc) and glucuronic acid (GlcUA) were present in varying proportion in Mucorales EPSs, depending on the strain. EPSs produced by the reference strain of *L. corymbifera* contain a high average amount of glucose (68%) unlike the clinical strain (2,5%) and *R. arrhizus* (0,5%) strain. These EPSs reacted immunologically with MM sera. However, antibodies directed against EPSs were present in most of the aspergillosis sera tested under our experimental conditions (about 25% of sera), suggesting a cross-reactivity to be taken into account in case of aspergillosis.

Conclusion. Inadequate antifungal treatment is recognized as an independent determinant of mortality in patients with MM, demonstrating the need of powerful and complementary tools to diagnose MM early. As sources of antibody production, EPSs seems to be promising candidates for developing a diagnostic assay for MM.

Key words: Mucormycosis; polysaccharides; diagnostic

Poster 19

Sialic acid biosynthesis is essential for network formation of iPSC-derived excitatory neurons

Rachel Mijdam[1], Raisa Veizaj[1], Hanneke Kwast[2], Marek Noga[2], Eline van Hugte[3], Chantal Bijnagte-Schoenmaker[3], Emma Dyke[3], Thomas Boltje[4], Nael Nadif Kasri[3,5] and Dirk Lefeber[1,2]

1. *Department of Neurology, Radboudumc, Donders Institute for Brain Cognition and Behavior, Nijmegen, the Netherlands*
2. *Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboudumc, Nijmegen, the Netherlands*
3. *Department of Human Genetics, Radboudumc, Donders Institute for Brain, Cognition and Behavior, Nijmegen, the Netherlands*
4. *Department of Organic Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen, the Netherlands*
5. *Department of Cognitive Neuroscience, Radboudumc, Donders Institute for Brain, Cognition and Behavior, Nijmegen, the Netherlands*

Multiple congenital disorders of glycosylation (CDG) are linked to sialic acid, a sugar commonly found in all human tissues, with the highest expression in the brain. Of these, only NANS-CDG is characterized by severe neurological symptoms. How changes in sialic acid biosynthesis lead to neurological symptoms is poorly understood. Here, we studied how the sialic acid biosynthesis pathway (SBP) influences neuronal network formation. To assess the requirement of sialic acid and the effect of disease causing variants in this pathway, excitatory neurons (iNeurons) were derived from control induced pluripotent stem cells (iPSC) treated with the sialyltransferase inhibitor (SiaFEt) and from three NANS-CDG patient-derived iPSC lines. Changes in sugar metabolite levels were followed by mass spectrometry-based metabolomics and spontaneous electrophysiological activity was monitored with micro electrode arrays. We found that SiaFEt inhibits the entire SBP and disturbs network formation in iNeurons, leading to a lower firing rate and shorter but more frequent bursts. NANS-CDG iNeurons presented with a delayed network formation. Metabolomics revealed high ManNAc-6P levels, but no reduction in sialic acid. Surprisingly, also the hexosamine biosynthesis pathway was affected, since GlcNAc-1P, GalNAc-1P and UDP-HexNAc levels were decreased. In conclusion, we demonstrate that disturbances in the SBP lead to abnormal neuronal network formation, but in NANS-CDG this cannot directly be related to a lack of sialic acid.

Poster 20

CLR-sequencing as a platform to study the selective immune induction of bacterial antibody responses at host barrier sites

Authors: Jasper Mol[1], Astrid Hendriks[1], Christoph Rademacher[2], Hilde Herrema[3], Nina van Sorge[1,4]

[1]Department of Medical Microbiology and Infection Prevention, Amsterdam UMC, location AMC, University of Amsterdam, Amsterdam, the Netherlands
[2]University of Vienna, Department of Pharmaceutical Sciences, Vienna, Austria
[3]Department of Experimental Vascular Medicine, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, The Netherlands
[4]Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam UMC, location AMC, Amsterdam, the Netherlands

Bacterial communities located at host barrier sites harbor species essential for human health but also species with pathogenic potential. Therefore, the human immune system has the intricate task to distinguish 'friend' from 'foe'. Although the host can naturally acquire antibody immunity to maintain microbiota symbiosis and defend against pathogens, the underlying molecular mechanisms and antibody targets are currently unknown. We focused on glycan structures in the bacterial cell wall as targets for specific glycan-binding receptors, specifically c-type lectin receptors (CLRs), on antigen presenting cells (APCs). APCs are local immune cells that constantly sample the environment through intercompartmental extension of their dendrites, which are densily covered with CLRs. Therefore, APCs are ideally positioned and equipped to probe bacteria-expressed glycans to build both local and systemic immunity, even in the absence of an infection. To examine this glycan-based selection, we have developed CLR-sequencing. This methodology exploits the natural glycan-binding properties of CLRs to identify interacting microbiota species through bacterial cell sorting and subsequent 16S rRNA sequencing. We established and optimized our experimental pipeline using fluorescently-labeled langerin and MGL and four different fecal microbiome donors. Langerin and MGL bound 9% and 4% of the fecal microbiome on average , respectively, but with substantial interindividual variation. These fractions are currently being sequenced to reveal the identity of the CLR-interacting species and comparison with unsorted and IgA-positive fractions taken from the same fecal samples. These results will provide insight into the mechanisms of local bacterial immune selection from a diverse community. The inclusion of IgA-positive bacteria from the same sample may connect this selection to local antibody immunity and give a better understanding of host-microbiota symbiosis.

Poster 21

Consequences of defective POMT-based protein O-mannosylation in the context of integrin-β1

Sina Noor

Defects in protein O-mannosylation lead to severe congenital muscular dystrophies collectively known as α-dystroglycanopathy. A hallmark of these diseases is the loss of the O-mannose-bound matriglycan on α-dystroglycan, which leads to reduced cell adhesion to the extracellular matrix and thereby diminishes the structural integrity of basement membranes. The initiation of O-mannosylation on α-dystroglycan is mediated by protein O-mannosyltransferase 1 and 2 (POMT1/2). Mutations in POMT1/2 have been associated with one type of congenital muscular dystrophy in human, known as Walker–Warburg syndrome (WWS). Our recent finding showed that in addition to α-dystroglycan, regulations of neuronal cadherin (N-Cdh) is also affected upon impaired O-mannosyl glycosylation. The existence of crosstalk among dystroglycan, cadherin and integrin via sharing the common cytoskeletal linkages, signaling molecules and adaptor protein has previously been reported. Therefore, in our present work, we investigated the functionality of highly N-glycosylated integrin in response to POMT-based defects of the O-mannosylation machinery. By using POMT1/2 knockout HEK293 cells, fibroblasts from WWS patients and by applying classic biochemistry, molecular and cell biology techniques in combination with transcriptomics and glyco(proteo)mics we revealed that defects in POMT1/2 activity affect integrin-β1 maturation and trafficking, which in turn negatively affect cell-matrix interactions, cell proliferation and cell migration. Moreover, alterations of N-glycan structures on over all proteins was observed in POMT1/2-deficient cells and that observed alterations correlate largely with impaired trafficking of integrin-β1. Altogether, our data demonstrate that in addition to impaired α-dystroglycan function, defective O-mannosylation also affects integrin performance implying a further level of complexity in the patho-mechanism of WWS caused by mutations in POMT1 and POMT2.

Poster 22

Studying congenital disorders of glycosylation in medaka fish using patient-based and conditional knock-down models

Kaisa Pakari[1,2], Joachim Wittbrodt[1], Thomas Thumberger[1]

[1] Centre for Organismal Studies (COS) Heidelberg Department of Developmental Biology, Heidelberg University, Germany
[2] Heidelberg International Graduate School of Molecular and Cellular Biology (HBIGS), Heidelberg University, Heidelberg, Germany

Rare human diseases like congenital disorders of glycosylation (CDG) are a growing group of metabolic disorders. Their pathophysiology is poorly elucidated and therapeutical approaches are limited. Null alleles of the glycosylation machinery enzymes are usually lethal. In CDG patients therefore mutations presumably cause global protein hypo-glycosylation which interferes with embryonic development and phenotypically is most prominent in neural and musculoskeletal tissues.

The most common forms of CDG are caused by compound heterozygous hypomorphic alleles of glycosylation enzymes. Phosphomannomutase 2 (PMM2) acts in the beginning of the glycosylation cascade and is the most common enzyme to harbor mutations in affected patients. It serves as an essential enzyme by providing Mannose-1-Phospate for the three main glycosylation types: *N*-glycosylation, *O*-glycosylation and *C*-mannosylation. The entire glycosylation machinery is evolutionarily highly conserved which allows generating translational model organisms. Especially fish models offer a great opportunity as the early embryogenesis can be studied through the extrauterine and transparent developing embryos. Two routes are followed to investigate the role of PMM2: patient-based genetic and conditional knock-down models in medaka fish (*Oryzias latipes*).

Patient-based mutations were generated with base editors due to the precise and predictable mode of action. Their applicability however is constrained by the required presence of a PAM motif in proper distance. We developed a novel two step one-shot approach termed “inception” to reach previously inaccessible base editing sites by *de novo* PAM generation for the introduction of patient-based mutations.

Alternatively, conditional knock-down of glycosylation enzymes on the protein level can mimic the hypomorphic activity reported from CDG patients. Along the line a nanobody-based degron systems can be used to manipulate the abundance of endogenous Pmm2 proteins *in vivo* by targeted degradation.

Both approaches are used to identify the sensitivity of important signaling pathways to differing levels of glycosylation during development and tissue maintenance.

Poster 23

Comparative HILIC-UPLC N-glycome analysis of immunoglobulin G from saliva and plasma

Barbara Radovani[1*], Gordan Lauc[2,3], Ivan Gudelj[1,2]

[1] Department of Biotechnology, University of Rijeka, Radmile Matejčić 2, Rijeka, Croatia
[2] Genos Glycoscience Research Laboratory, Borongajska cesta 83h, Zagreb, Croatia
[3] Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, Zagreb, Croatia

Immunoglobulin G (IgG) is the most abundant antibody in the blood and plays a critical role in host immune defence against infectious pathogens. IgG glycosylation is known to modulate IgG effector functions and is involved in the development and progression of disease. Because the reactivity of salivary IgG mirrors that of plasma IgG, saliva is an attractive alternative to blood for studies in which antibody analysis is intended to provide an indication of a subject's immune status. Therefore, in this study, we described a rapid and effective method for comparative N-glycome analysis of IgG from human plasma and saliva samples. Plasma and saliva were collected from 9 healthy volunteers within a 2-hour time window. IgGs were affinity-purified from the two biofluids with protein G-agarose beads and then denatured and enzymatically deglycosylated. The released N-glycans were analysed by ultra-high performance liquid chromatography with hydrophilic interaction analysis (HILIC-UPLC). IgG from saliva exhibited reduced galactosylation and sialylation compared with IgG from plasma, whereas core fucosylation remained unchanged. The slightly proinflammatory profile of salivary IgG may be due to higher exposure to pathogens compared with plasma IgG. This study provides an additional tool for the analysis of salivary IgG glycosylation and highlights its potential as a surrogate biomarker for the study of plasma IgG.

Poster 24

The transcriptional landscape of glycosylation-related genes in cancer

Ernesto Rodriguez, Dimitri Lindijer, Sandra van Vliet, Juan J. Garcia Vallejo, Yvette van Kooyk

Amsterdam UMC location Vrije Universiteit Amsterdam, Molecular Cell Biology and Immunology, De Boelelaan 1117, Amsterdam, Netherlands

Cancer Center Amsterdam, Cancer Biology and Immunology, Amsterdam, The Netherlands

Amsterdam institute for Infection and Immunity, Cancer Immunology, Amsterdam, The Netherlands

Abstract

Glycosylation is a complex metabolic process that leads to the synthesis of glycan structures that can be attached to proteins, lipids or RNA. Changes in glycosylation patterns have been associated with malignant transformation and clinical outcome in several types of cancer, although no comprehensive analysis has been performed in a pan-cancer setting. Here, we performed an extensive pan-cancer analysis of the expression of glycosylation related genes, using publicly available bulk and single cell transcriptomic data sets from tumor samples and cancer cell lines. We identified genes and pathways associated with different tumor types, which can be used as novel diagnostic biomarkers. As a proof of concept, we studied the biomarker potential of Galectin-7, found to be specifically expressed in Squamous cell carcinomas (SCC). In a small cohort of Lung cancer patients, serum levels of Galectin-7 were elevated in SCC but not in Adenocarcinomas. Moreover, we characterized the contribution of different cell types to the tumor glycosylation patterns by integrating and analyzing single cell RNA-seq data from 14 different tumor types, which led us to identify cancer cells as the main contributors to the specific tumor glyco-codes. Our results show that the genes and pathways expressed by cancer cells are influenced by the cell of origin and the oncogenic pathways that led to malignant transformation. Finally, we described the association of different glycosylation-related genes and pathways with survival. Altogether, our results represent an extensive transcriptomic analysis of glycosylation pathways in cancer and can serve as a resource for future research.

.

Poster 25

Use of metabolic oligoengineering to decipher the regulation of complex-type glycosylation by *O*-GlcNAcylation in human colon cells

Jodie Scache[1], Vincent Rigolot[2], Cédric Lion[2], Christophe Biot[2], Tony Lefebvre[1], Anne-Sophie Vercoutter-Edouart[1]

[1] "Regulation of molecular and cellular mechanisms by *O*-GlcNAcylation" Team

[2] "Chemical glycobiology " Team

Unit of Structural and Functional Glycobiology, UGSF, CNRS, UMR 8576 - University of Lille, France

Abnormal *O*-GlcNAcylation and overexpression of *O*-GlcNAc transferase (OGT) have been reported in various cancers, contributing to tumor growth. We previously showed that OGT extinction by siRNA alters the adhesion and migration properties of colon cancer cells and slightly affects the cell surface glycosylation. To go further, we apply metabolic oligosaccharide engineering (MOE) using synthetic sialic acid precursors to label neosynthesized and cell surface glycoproteins (GPs) that could be affected by abnormal *O*-GlcNAc levels. We first compared the metabolic incorporation of various alkyne- or azide-modified ManNAc or SiaNAc analogs to select the most appropriate chemical reporter in the human colon cells CCD841CoN, HT29 and HCT116. Then, MOE strategy using ManNAz is applied in cells in which *O*-GlcNAc cycling is perturbed by either inhibition or silencing of OGT or *O*-GlcNAcase (OGA). After 24h of incubation with ManNAz and Copper-catalyzed azide-alkyne cycloaddition (CuAAc), Western-blot analysis shows a significant increase of labeled GPs in siOGT condition in the two cancer cell lines but not in non-cancerous CCD841CoN cells, while OGT inhibition seems to affect the labeling of GPs only in HT29 cells. We are now doing pulse-chase experiments to monitor the trafficking of sialylated GPs into cells by confocal imaging. Our preliminary results suggest that OGT inhibition or silencing reduces the trafficking of tagged sialylated GPs within intracellular vesicles. Further experiments are needed to have a better understanding as to how OGT may regulate post-Golgi traffic of GPs.

Poster 26

CHARACTERISATION OF A GENE CLUSTER INVOLVED IN *ASPERGILLUS FUMIGATUS* ZWITTERIONIC AF3C GLYCOSPHINGOLIPID SYNTHESIS

Carla I.I. Seegers[1], Isabel Ramón Roth, Patricia Zarnovican, Falk F.R. Buettner and Françoise H. Routier[1]

[1] Institute for Clinical Biochemistry, OE4340, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

The human pathogenic fungus *Aspergillus fumigatus* is a saprotrophic mold that can be found in soil worldwide and plays an important role for the recycling of carbon and nitrogen sources. While it is no threat for healthy individuals, this fungus creates a set of diseases ranging from allergic sinusitis to lethal invasive mycosis in immunocompromised patients. A variety of drugs are available for treatment against infections with *A. fumigatus*, however, most of them show severe side effects such as nephrotoxicity. Over the last decades, new azoles improved the treatment of invasive aspergillosis but azole resistant strains raise a significant problem. Therefore, elucidating the biosynthesis and function of unique glycostructures found in *A. fumigatus* plays a major role in identifying new drug targets to develop new classes of antifungal drugs. In this work, we analysed a cluster of 5 genes and described its implication in the biosynthesis of the unique glycosphingolipid Af3c, a zwitterionic Manα1,3Manα1,6GlcNα1,2-IPC. Each of the genes AFUA_8G02040 to AFUA_8G02090 was deleted in *A. fumigatus* using CRISPR Cas9 homologous directed repair and the resulting glycolipids were analysed by MALDI-Mass spectrometry. The obtained glycolipid profiles support the role of the gene products as a UDP-GlcNAc transporter (NstA), an α1,2-N-acetylglucosaminyltransferase (GntA), a GlcNAc de-N-acetylase (GdaA), an α1,6- and an α1,3-mannosyltransferase (OchC and ClpC). Moreover, successive introduction of the genes encoding GntA, GdaA, OchC and ClpC in the yeast *Saccharomyces cerevisiae* enabled the reconstitution of the Af3c biosynthetic pathway. Finally, we show that the glycosphingolipid Manα1,3Manα1,6GlcNα1,2-IPC is required for full virulence of *A. fumigatus* in a *Galleria mellonella* infection model.

This work was supported by the German Research Foundation DFG (EXC 2155 –390874280)

Poster 27

The glycosyltransferase SCWPI affects the composition and function of the rhamnose-rich cell wall polysaccharides of *Streptococcus suis*

Yao Shi[1 2] , Thomas Roodsant[2 3] , Boas C. L. van der Putten[2 4] , C. Coral Domínguez-Medina[2] , Kees C. H. van der Ark[2 3] , Arjan Stegeman[1] , Constance Schultsz[2 3] , Nina M. van Sorge[2 4] , Lindert Benedictus[1]

1. Department of Population Health Sciences, Utrecht University, Utrecht, The Netherlands
2. Department of Medical Microbiology and Infection Prevention, Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands
3. Department of Global Health-Amsterdam Institute for Global Health and Development, Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands
4. Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands

The cell walls of Streptococcus spp. are decorated with cell wall-associated polysaccharides, including capsular polysaccharides (CPS) and rhamnose-rich cell wall polysaccharides (RhaCWP). RhaCWP are abundant structures, important for bacterial homeostasis, growth, and cell division. Furthermore, they are also important for Streptococcal host cell adhesion, resistance to antibacterial peptides, evading phagocytosis, and antibiotic resistance. However, in the zoonotic and major pig pathogen *Streptococcus suis*, RhaCWP have not been studied. In a bioinformatic analysis of the putative RhaCWP biosynthesis gene cluster of *S. suis* reference strain P1/7, we identified a 5-gene rhamnose and a 14-gene RhaCWP biosynthesis gene cluster. Population analysis of 1719 *S. suis* whole genome sequences revealed that the rhamnose biosynthesis cluster was highly conserved, but there was considerable diversity in the RhaCWP gene cluster. In 11.7% of isolates, the gene content of the RhaCWP gene cluster was variable, whilst in the remaining isolates, the genetic organization was conserved with allelic diversity restricted to two putative glycosyltransferases.

One of the glycosyltransferases, SCWPI, has nonsynonymous mutations in the putative functional domain in the pathogenic ST-16 and ST-20 lineages. Plant lectin binding assays of *SCWPI* knock-out mutants and homologous and heterologous complemented strains showed that SCWPI transfers a different sugar to the RhaCWP in the ST16/ST20 lineages compared to CC1 lineages. Interestingly, *SCWPI* knock-out mutants showed reduced growth and increased susceptibility to oxidative stress, while maximal bacterial cell density and chain length were unaffected compared to wild-type strains. Our results indicate that the genetic variation in *SCWPI* results in differences in the sugar composition of RhaCWP and *S. suis* requires RhaCWP for intrinsic physiology. Planned NMR analyses will elucidate the function of *SCWPI* in the RhaCWP biosynthesis pathway and additional phenotypical assays will be performed to study the role of *SCWPI* in cell division and virulence.

Poster 28

Functionality of O-mannosylation in an ALG3 deficient HEK cell model

Daniel Sturm

Abstract: What is the functional importance of N-glycosylation sites on both POMT (protein-O-mannosyl-transferase) proteins - the enzymes that work together in a complex to initiate classic O-mannosylation on specific target proteins? To address this question, an N-glycosylation deficient ΔALG3 (alpha-1,3-mannosyltransferase) HEK cell line has been generated to evaluate the effects on POMTs and O-mannosylation. ALG3 is active in the early N-glycosylation pathway, where it is involved in the biosynthesis of the lipid-linked oligosaccharide, that later gets transferred on nascent polypeptide chains. When looking at ALG3 congenital disorder of glycosylation (ALG3-CDG) patient-derived fibroblasts, the N-glycans transferred on POMT1 and POMT2 are partly immature. This can directly be linked to a reduced enzymatic activity of ALG3. To analyze this defect in more detail, we have created ΔALG3 HEK cells, where ALG3 activity is completely abolished. Taking advantage of this cellular model, we started to characterize the effects of aberrant N-glycosylation on POMT O-mannosyltransferases. In this cell line, reduced abundance of POMTs carrying only immature N-glycans might hint to an impaired POMT-mediated O-mannosylation process.

Poster 29

The SARS-CoV-2 N-terminal spike domain is primed to engage 9-*O*-acetylated 2-8-linked sialic acids

Ilhan Tomris[1], Luca Unione[2], Linh Nguyen[3], Pouya Zaree[1], Kim M. Bouwman[1], Lin Liu[4], Zeshi Li[1], María Ríos Carrasco[1], Roosmarijn van der Woude[1], Anne Kimpel[1], Mirte Linthorst[1], Enrico Verpalen[1], Jelle A. Fok[1], Tom Caniels[5,6], Rogier W. Sanders[5,6,7], Balthasar A. Heesters[1], Roland J. Pieters[1], Jesus Jimenez-Barbero[2], John Klassen[3], Geert-Jan Boons[1,4] and Robert P. de Vries[1*]

1 Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands
2 CICbioGUNE, Basque Research & Technology Alliance (BRTA), Bizkaia, Spain
3 Department of Chemistry, University of Alberta, Edmonton, Canada
4 Complex Carbohydrate Research Center, University of Georgia, Athens, USA.
5 Department of Medical Microbiology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands.
6 Amsterdam Institute for Infection and Immunity, Infectious Diseases, Amsterdam, the Netherlands.
7 Department of Microbiology and Immunology, Weill Medical Center of Cornell University, New York, USA

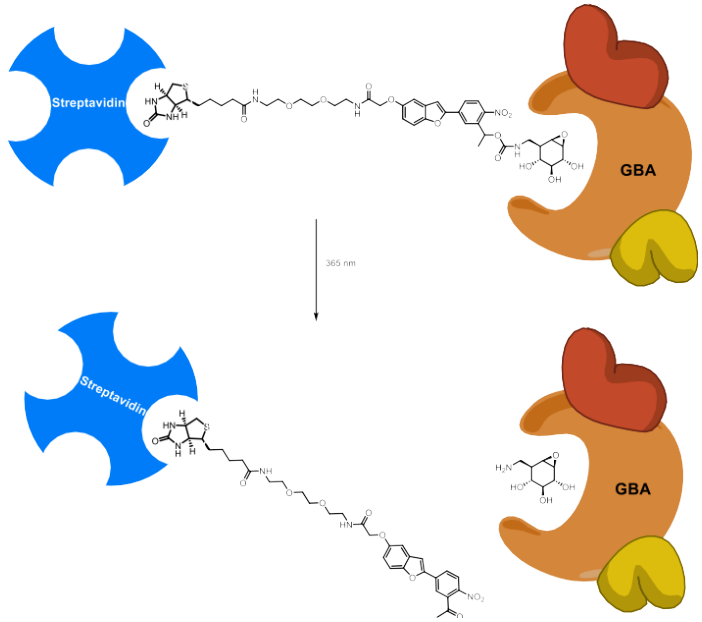
Abstract
SARS-CoV-2 viruses engage ACE2 as a functional receptor with their spike protein. This spike protein contains the receptor-binding domain but also an N-terminal domain that for other coronaviruses contains a glycan-binding cleft. However, we were never able to pick up glycan-binding for the SARS-CoV-2 NTD protein besides its interaction with heparan sulfate. This NTD domain is under antigenic pressure resulting in amino acid changes in Variants of Concern, which is also an indication of functionality. To analyze if the Variants of Concern gained a glycan-binding modality, we created trimeric fluorescent NTD proteins to analyze possible receptor binding properties on Vero e6 cells. To our surprise, the Beta NTD gained binding to cells which was abrogated by sialidase pretreatment. We identified a putative 9-*O*-acetylated sialic acid by glycan microarray analyses and confirmed this specificity by catch-and-release ESI-MS, STD NMR analyses, and a graphene-based electrochemical sensor.

Poster 30

Photocleavable ABPs for Native Pull Down Assays

N.E. Trenkler, M.J. van der Graff, S.I. van Kasteren, M. Artola, J.M.F.G. Aerts and H.S. Overkleeft Dept of Bio-organic synthesis, Leiden Institute of Chemistry, Leiden University, The Netherlands
Correspondence: n.e.trenkler@lic.leidenuniv.nl

Lysosomal storage diseases, like Gaucher and Pompe disease, are caused by the accumulation of metabolites (glucosylceramide and glycogen, respectively) in the lysosome due to impairment of their degrading enzymes Glucocerebrosidase and Lysosomal α-glucosidase [1,2]. For a better understanding of the diseases, the affected enzymes and their biological environment are investigated extensively. Identifying proteins that are interacting with these enzymes, for example activating or stabilizing them, could give insight in the enzymes' mechanisms and activated downstream pathways. In this work we identify and characterize these interacting proteins via a native pull down assay using photo-cleavable activity-based probes.



1. Aerts, J. M. *et al.* Lysosomal Storage Diseases. For Better or Worse: Adapting to Defective Lysosomal Glycosphingolipid Breakdown. *eLS* 1–13 (2017). doi:10.1002/9780470015902.a0027592
2. Fukuda, T. & Roberts, A. Acid Alpha-Glucosidase Deficiency (Pompe Disease). (2007).

Poster 31

32nd Joint Glycobiology Meeting:
Eline Visser, PhD Candidate Boltje Research Group, RU Nijmegen

Abstract:
Studying metabolic inhibitors of glycosylation for potential therapeutic use
Glycosylation, the addition of glycans on about half of all proteins and often found on the cell surface, impacts a myriad of physiological and pathological processes. Looking into methods to alter glycosylation pathways might emerge as potential therapeutic avenues. One method to do this is by using metabolic inhibitors. Metabolic inhibition of sialylation and fucosylation, two glycan building blocks often found at the outermost location, was first shown a decade ago (1): peracetylated 3-fluoroneuraminic acid and 2-fluoro-fucose (2FF) can passively enter mammalian cells, after which they are metabolized similarly to the sugars they mimic. However, due to addition of the fluor, the enzymes normally incorporating the sugar building blocks to glycans cannot incorporate them anymore. We improved these inhibitors in our group. For sialic acid, modifications on the C5-position resulted in a more efficient conversion into the activated CMP-sugar and thus a higher potency (2). For fucose we found that incorporating a protected phosphate-group allows the inhibitor to skip a step in the metabolic pathway, enhancing the potency (3). These inhibitors are interesting tools for further use in studying sugar metabolism and the roles in health and disease, and they are potential therapeutic candidates.

1. Rillahan CD, Antonopoulos A, Lefort CT, Sonon R, Azadi P, Ley K, et al. Global metabolic inhibitors of sialyl- and fucosyltransferases remodel the glycome. Nat Chem Biol. 2012;8(7):661-8.
2. Heise T, Pijnenborg JFA, Büll C, van Hilten N, Kers-Rebel ED, Balneger N, et al. Potent Metabolic Sialylation Inhibitors Based on C-5-Modified Fluorinated Sialic Acids. J Med Chem. 2019;62(2):1014-21.
3. Pijnenborg JFA, Visser EA, Noga M, Rossing E, Veizaj R, Lefeber DJ, et al. Cellular Fucosylation Inhibitors Based on Fluorinated Fucose-1-phosphates. Chemistry. 2021;27(12):4022-7.*

Poster 32

Development of a proximity assay to detect proteins that bind to specific heparan sulfate sequences

Thierry van Wessel[1], Lars A. A. Damen[1], Elly Versteeg[1], Bart Straten[1], Prof. Dr. David G. Fernig[3], Dr. Nelleke Spruijt[2], Dr. Toin H. van Kuppevelt, Dr[1]. Willeke F. Daamen[1]

1. *Dept. of Biochemistry, Radboud Institute for Molecular Life Sciences, Radboud university medical center, The Netherlands*
2. *Dept. of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Oncode Institute, Radboud University Nijmegen, The Netherlands*
3. *Dept. of Biochemistry, Faculty of Health and live sciences, University of Liverpool, United Kingdom*

Heparan sulfate (HS) is a linear polysaccharide with high structural and functional diversity. This negatively charged molecule resides intracellularly, on cell membranes and in the extracellular matrix. Differences in HS chains arise from several modifications, like epimerization and sulfation. These modifications are not equally distributed across the chain, allowing sulfation motifs to form. Identifying the function of HS motifs is challenging due to a lack of analytical tools and large variations between each HS molecule. Single chain variable fragment (scFv) antibodies can recognize specific sulfation motifs and may be used to localize specific HS motifs in tissues. As different sulfation motifs have been detected in diseased and normal tissue, localization of certain motifs and their interactomes may lead to the identification of new therapeutic targets. Many proteins have been described that interact with HS, but since HS is highly variable, it is difficult to identify which HS motifs interact with a particular protein.

Here we introduce a new approach for the identification of proteins in proximity to specific HS motifs. Anti HS scFv antibodies were fused to a peroxidase (scFv-APEX2) creating a fusion protein which was shown to preserve its epitope specificity. As a model system, rat kidney sections (rich in HS epitopes) were incubated with the fusion protein, and proteins in proximity to HS were labeled with biotin by the peroxidase using biotinyl tyramide and hydrogen peroxide as substrates.

After scraping off the sections and extraction of proteins, streptavidin resins were applied to isolate biotinylated proteins. On-bead trypsin digestion will be used to retrieve peptides which will be analyzed by LC-MS/MS.

Acknowledgements

This study was financially supported by HS-SEQ H2020 FETOPEN RIA 899687 and ArrestAD H2020 FETOPEN RIA 737390.

Poster 33

Activity-based probes to unravel the the mode of action of the Pseudomonas aeruginosa biofilm degrading hydrolase PslG

Gijs Ruijgrok, Leiden University

Pseudomonas aeruginosa is a Gram-negative bacterium that mainly infects immunocompromised and cystic fibrosis patients. P. aeruginosa can produce a biofilm, that protects it against antibiotics and form the host immune system. The biofilm contains different exopolysaccharides which are key for its protective capacities. The main polysaccharide, Psl, is built up from pentasaccharide repeats and acts as a ‘molecular glue’ for the bacteria. P. aeruginosa also produces a glycoside hydrolase (PslG) which can degrade Psl. It has been shown that addition of PslG can disrupt the Psl biofilm to negate antibiotic resistance of P. aeruginosa.1,2 The mode of action of PslG however PslG remains elusive which hinders further developments of therapeutics.3 Here we elucidate the mode of action of PslG using a set of Psl-type cyclophellitol derivates, that can act as covalent inhibitors. The probes represent frame-shifted fragments of the Psl oligosaccharide to unravel where and how PslG cleaves the exopolysaccharide. The cyclophellitol probes were used in activity-based protein profiling assays, through which we were able to determine the cleavage site of PslG. The results will aid in a methodology for discovering more biofilm disrupting glycoside hydrolases.

1. Yu S, Su T, Wu H, et al. Cell Research. 2015;25(12):1352-1367. doi:10.1038/cr.2015.129
2. Baker P, Whitfield GB, Hill PJ, et al. Journal of Biological Chemistry. 2015;290(47):28374-28387. doi:10.1074/JBC.M115.674929
3. Su T, He J, Li N, Liu S, Xu S, Gu L. Frontiers in Microbiology. 2020;11(May):1-9. doi:10.3389/fmicb.2020.00760

Poster 34

Charting the proteoform landscape of the serum proteins by high-resolution native mass spectrometry

Dario A. T. Cramer[1,2], Vojtech Franc[1,2], Tomislav Caval[1,2], Albert J. R. Heck[1,2 #].

[1]Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Science, Utrecht University, Padualaan 8, Utrecht, 3584 CH, The Netherlands

[2]Netherlands Proteomics Centre, Utrecht University, Padualaan 8, Utrecht, 3584 CH, The Netherland

Keywords (max 3): native mass spectrometry, glycoproteomics, serum

Abstract

Many serum proteins are glycosylated, among them several already being annotated as biomarkers. So far, most methods for analysis of serum glycoproteins have used either glycan or glycopeptide centric mass spectrometry-based approaches, which provide excellent tools for glycan analysis, but often neglect undefined or unknown glycosylation or other co-occurring modifications. High-resolution native mass spectrometry is a technique for the analysis of intact glycoproteins, allowing qualitative and quantitative observation of all modifications present on a glycoprotein. So far, this approach has been used mostly on just one specific protein at the time. We introduce a method capable of isolating over 20 (glyco)proteins in parallel from serum, covering a mass range between 30 and 200 kDa.Prior to the analysis about 100 μL of serum is first depleted of 3 of its most abundant proteins, whereafter serum (glyco)proteins are fractioned over tandem an- and cationic ion-exchange columns. We follow the IEX-based fractionation of these proteins with an in-depth characterization using high-resolution native mass spectrometry. Over 20 serum glycoproteins could be fractionated and their proteoform profiles analyzed using native mass spectrometry portraying a wide variety of masses, modifications and functions. Fractionated proteins include protease inhibitors, complement components, transporter proteins and more. The proteoform profiles of four selected proteins of interest, *i.e.* alpha-1-antitrypsin, ceruloplasmin, hemopexin and complement protein C3, are characterized further in-depth. This includes, among others, the annotation of N- and O-glycans, protein cysteinylation, cleavage induced protein activation as well as the detection of co-occurring genetic variants. Finally, we expand our approach to a small sample set of serum samples obtained from healthy and diseased individuals. We qualitatively and quantitatively monitor the changes in proteoform profiles of ceruloplasmin and reveal a substantial increase in fucosylation and glycan occupancy in patients with late-stage hepatocellular carcinoma and pancreatic cancer, when compared to controls.

Poster list

Poster #	Last name
1	Allen
2	Armony
3	Van Bakel
4	Blatitzki
5	Bruijns
6	Charlet
7	Clarisse
8	Coccimiglio
9	Conte
10	De Koster
11	Driessen
12	Freville
13	Friedrich
14	Gudelj
15	Balsollier
16	Kayser
17	Laplanche
18	Lecointe
19	Mijdam
20	Mol
21	Noor
22	Pakari
23	Radovani
24	Rodriguez
25	Scache
26	Seegers
27	Shi
28	Sturm
29	Tomris
30	Trenkler
31	Van Wessel
32	Visser
33	Ruijgrok
34	Cramer
35	

Albers	Hannover Medical School	albers.marco@mh-hannover.de
Allen	University of Southampton	joel.allen@soton.ac.uk
Althoff	Université de Lille & KU Leuven	charlotte.althoff.etu@univ-lille.fr
Alvarado Melendez	Utrecht University	e.i.alvaradomelendez@uu.nl
Armony	RadboudUMC	gad.armony@radboudumc.nl
Armstrong	Leiden Institute of Chemistry	z.w.b.armstrong@lic.leidenuniv.nl
Attabey	Amsterdam UMC	t.atabey@amsterdamumc.nl
Bakel	Quadram Institute Bioscience	Wouter.Van-Bakel@quadram.ac.uk
Bakker	Hannover Medical School	bakker.hans@mh-hannover.de
Balsollier	Utrecht University	c.balsollier@uu.nl
Balzarini	Amsterdam UMC	f.balzarini@amsterdamumc.nl
Beimdiek	Hannover Medical School	beimdiek.julia@mh-hannover.de
Blawitzki	Heinrich-Heine Universität Düsseldorf	luca-cesare.blawitzki@hhu.de
Bortolus	UGSF - U1285 - CHRU de Lille	clovis.bortolus@inserm.fr
Bouckaert	CNRS - Laboratoire UGSF	julie.bouckaert@univ-lille.fr
Brand	Hannover Medical School	brand.smilla@mh-hannover.de
Breeuwsma	Utrecht University	m.breeuwsma@uu.nl
Büll	Radboud University, IMM	christian.bull@ru.nl
Calzari	Radboud University Nijmegen	matteo.calzari@ru.nl
Charlet	CNRS UMR 8576 UGSF - U1285 - CHRU de Lille	rogatien.charlet@inserm.fr
Clarisse	UGSF UNIV DE LILLE	charlotte.clarisse@univ-lille.fr
Coccimiglio	Amsterdam UMC, location VUmc	m.b.coccimiglio@amsterdamumc.nl
Conte	RadboudUMC	federica.conte@radboudumc.nl
Coulon	CNRS UMR 8576 UGSF - U1285 - CHRU de Lille	pauline.coulon@chu-lille.fr
Crispin	University of Southampton	
de Vries	Utrecht University	r.vries@uu.nl
Derking	Amsterdam UMC	r.derking@amsterdamumc.nl
Doelman	Leiden University	w.doelman@lic.leidenuniv.nl
Driessen	Heinrich Heine University	Marc.Driessen@HHU.de
Durin	UGSF-UMR 8576 Université Lille/CNRS	zoe.durin.etu@univ-lille.fr
Eggink	RIVM	dirk.eggink@rivm.nl
Feldhof	Heinreich-Heine-Universität	melina.feldhof@hhu.de
Friedrich	MHH	friedrich.alina@mh-hannover.de
Gerling-Driessen	Heinrich Heine University Duesseldorf	ulla.gerling-driessen@hhu.de
Goossens	Amsterdam UMC	l.kruijssen@amsterdamumc.nl
Groux-Degroote	UGSF UNIVERSITE DE LILLE	sophie.groux-degroote@univ-lille.fr
Gudelj	University of Rijeka	ivan.gudelj@uniri.hr
Haan	University of Copenhagen	ndehaan@sund.ku.dk
Hafkenscheid	Technical University of Denmark	lihaf@dtu.dk
Hardiville	UGSF UNIV DE LILLE	stephan.hardiville@univ-lille.fr
Heming	Leiden University	j.j.a.heming@lic.leidenuniv.nl
Hendriks	Amsterdam UMC	a.hendriks2@amsterdamumc.nl
Hoang Son	Otto-von-Guericke-Universität	son@mpi-magdeburg.mpg.de

Hoffmann	Heinrich-Heine-Universität Düsseldorf	miriam.hoffmann@hhu.de
Hokke	Leiden University	
Hornikx	Radboud University	daniel.hornikx2@ru.nl
Huang	Radboudumc	Sjanie.huang@radboudumc.nl
Jäck	Heinrich-Heine University	nijae102@hhu.de
Juárez Osorio	Max Planck Institute	juarez@mpi-magdeburg.mpg.de
Kamerling	Utrecht University	j.p.kamerling@uu.nl
Kasprowicz	UGSF UNIVERSITE DE LILLE	angelina.kasprowicz@univ-lille.fr
Kayser	Heinrich Heine University Dusseldorf	alisa.kayser@hhu.de
Kooyk	Free University of Amsterdam	
Koster	Universiteit Leiden	n.de.koster@lic.leidenuniv.nl
Laplanche	Quadram Institute Bioscience	victor.laplanche@quadram.ac.uk
Latousakis	Quadram Institute Bioscience	dimitris.latousakis@quadram.ac.uk
Lecointe	UGSF - U1285 - CHRU de Lille	lecointe.karine@gmail.com
Lefeber	Utrecht University	
Lenartowicz	Utrecht University	p.lenartowicz@uu.nl
Mank	Danone Nutricia Research	marko.mank@danone.com
Mijdam	Radboudumc	rachel.mijdam@radboudumc.nl
Mohamed	Gothenburg university	Kay93Mohamed@outlook.com
Mol	Amsterdam UMC	j.m.a.mol@amsterdamumc.nl
Moscovitz	Max Planck Institute	oren.moscovitz@mpikg.mpg.de
Noor	COS, University of Heidelberg	sina.ibne.noor@cos.uni-heidelberg.de
Ong	Utrecht University	j.y.ong@uu.nl
Padler-Karavani	Tel Aviv University	vkaravani@tauex.tau.ac.il
Pakari	AG Wittbrodt/Thumberger - COS Heidelberg	kaisa.pakari@cos.uni-heidelberg.de
Post	RadboudUMC	merel.post@radboudumc.nl
Psomiadou		venetia.psomiadou@ru.nl
Radovani	University of Rijeka	barbara.radovani@uniri.hr
Rahm	Radboudumc	Moritz.Rahm@radboudumc.nl
Rigolot	UGSF UMR 8576 CNRS - Université de Lille	vincent.rigolot.etu@univ-lille.fr
Ríos Carrasco	Utrecht University	mariarioscarrasco@gmail.com
Rodriguez	MCBI - Amsterdam UMC	j.rodriguezcamejo@amsterdamumc.nl
Routier	Hannover Medical School	Routier.Francoise@mh-hannover.de
Ruijgrok	Leiden University	g.ruijgrok@lic.leidenuniv.nl
Sanders	Amsterdam UMC	r.w.sanders@amsterdamumc.nl
Scache	UGSF UNIV DE LILLE	jodie.scache@univ-lille.fr
Scherpenzeel	GlycoMScan B.V.	monique.vanscherpenzeel@glycomscan.com
Seegers	Hannover Medical School (MHH)	seegers.carla@mh-hannover.de
Shamorkina	Utrecht University	t.m.shamorkina@uu.nl
Shi	Utrecht University	y.shi1@uu.nl
Snijder	Utrecht University	j.snijder@uu.nl

Sorge	Amsterdam UMC	n.m.vansorge@amsterdamumc.nl
Spruit	Utrecht University	c.m.spruit@uu.nl
Stahl	Nutricia Research	bernd.stahl@danone.com
Stapels	Utrecht University	d.a.c.stapels@uu.nl
Steuten	Leiden Institute of Chemistry	k.steuten@lic.leidenuniv.nl
Straten	Utrecht University	b.f.straten@uu.nl
Strijbis	Utrecht University	
Sturm	COS, University of Heidelberg	daniel.sturm@cos.uni-heidelberg.de
Tamminga	Amsterdam UMC	s.m.tamminga@amsterdamumc.nl
Temming	Amsterdam UMC, location AMC	a.r.temming@amsterdamumc.nl
Tomris	Universiteit Utrecht	i.tomris@uu.nl
Trenkler		n.e.trenkler@lic.leidenuniv.nl
Uslu	Universiteit Utrecht	e.uslu@uu.nl
Vercoutter-Edouart	UGSF UNIV DE LILLE	anne-sophie.vercoutter@univ-lille.fr
Verpalen	Utrecht University	e.c.j.m.verpalen@uu.nl
Visser	Radboud University	Eline.Visser@ru.nl
Vliegenthart	Utrecht University	j.f.g.vliegenthart@uu.nl
Vries	Utrecht University	
Walvoort	Groningen University	
Wessel	Thierry van Wessel	thierry_wessel@hotmail.com
Wessels	Radboud University	
Wuhrer	Leiden University Medical Center	m.wuhrer@lumc.nl
Zhang		z.zhang2@uu.nl