BEILSTEIN SYMPOSIUM

Unparalleled Diversity and Functionality of the Glycome

Beilstein Glyco-Bioinformatics Symposium 2023

June 20–22, 2023
Dom Hotel Limburg
Limburg (Lahn), Germany

Beilstein-Institut and Open Science

The non-profit Beilstein-Institut is one of the most respected organizations in the communication and dissemination of high-quality information in chemistry. Since 1951, when the foundation was established by the Max Planck Society, we have been fulfilling our mission to support the scientific community by providing high-quality information that is essential for research.

Our role has evolved over the years: from the production of the Beilstein Handbook and Database, to being one of the first open access journal publishers in chemistry, to host of interdisciplinary symposia and webinars and supporter of open data initiatives. We believe that free access to scientific research results, giving everyone in the world an equal chance to participate in the exchange of experimental findings and data, is the best way to advance science.

Open Science is a new paradigm to scientific research. It is based on cooperation and creates new ways to disseminate information and broaden knowledge through digital technologies and new collaborative tools. It aims to make the primary outputs of publicly funded research results – publications (open access) and the research data (open data) – publicly accessible in digital format with no or minimal restriction.

The Beilstein-Institut supports open science and makes the results of its projects freely available to the scientific community, which is an essential contribution to the foundation's mission to advance the chemical and related sciences. All journal articles, conference proceedings, webinars and videos are open access to allow the worldwide, unhindered sharing and exchange of ideas. This allows scientists, students, educators and the public the opportunity to inform themselves of the latest developments in research and to build on these ideas to further advance scientific knowledge.

Our two diamond open access journals, the *Beilstein Journal of Organic Chemistry* and the *Beilstein Journal of Nanotechnology*, which we fully fund, have no fees for authors or readers. Both journals are produced and managed by the Beilstein Editorial Office team, who work together with a global scientific network of experts that are responsible for the peer review. In 2015, the Beilstein Journals were awarded the DOAJ Seal which recognizes the exceptionally high level of publishing standards and best practices adhering to these journals. In addition, as our journals are Plan S compliant, they meet all technical specifications and policy requirements as outlined by cOAlition S. This means publishing with our journals will ensure compliance with all major funding agency publishing mandates including NIH, NSF, UKRI, ERC, DFG, RCUK, European Commission and Wellcome Trust.

Both journals publish thematic issues on subjects of high contemporary interest; these are often edited by guest editors, further expanding our network and outreach.

Launched in April 2019, a further addition to our publishing platform is the Beilstein Archives. This is the preprint server for the Beilstein journals. During the manuscript submission process, authors have the option to request that the manuscript is posted as a preprint, which is the version of the manuscript before peer review. Our preprints are posted on average within two days, allowing authors to rapidly disseminate their research results and ensuring that they claim priority for their work. Currently, over 25% of our authors select the preprint option.

The Beilstein-Institut runs two data standards projects: STRENDA which is concerned with the reporting of enzymology data and MIRAGE which is working on guidelines for the reporting of glycomics experimental results. Both of which are now widely accepted and acknowledged by the scientific community.

The direct interaction and the exchange of thoughts and ideas between scientists are supported by a program of regularly hosted symposia. These international meetings are organized by the Beilstein-Institut and cover a variety of topics ranging from organic chemistry and biochemistry to nanotechnology and open science as well as interdisciplinary meetings on contemporary topics.

The Beilstein-Institut has been hosting symposia since 1988. Each meeting is always a unique event, and the lively and intense exchange of thoughts and ideas of the participants turn it into a memorable and lasting experience. The number of participants is usually limited to around 50 and the program is designed specifically to allow sufficient time for discussions. The talks also provide a framework and catalyse discussions which often go on into the night and have led to subsequent cooperation projects. The resulting exchange between researchers, at all stages of their careers, is the underlying goal of the meeting and gives the Beilstein Symposium their unique character.

Upcoming events in this year are:

Beilstein Enzymology Symposium 2023 *From Molecular Mechanisms to High-Performance Systems*	Scientific Program: Edda Klipp, Joelle Pelletier, Jürgen Pleiss and Carsten Kettner
September, 12–14, 2023, Rüdesheim, Germany.	www.enzymology.beilstein-symposia.org

4th EnzymeML Workshop

September 26–27, 2023, Rüdesheim, Germany

Scientific Program:
Jürgen Pleiss and Carsten Kettner

https://www.beilstein-institut.de/en/projects/strenda/meetings/4th-enzymeml-workshop/

Beilstein Nanotechnology Symposium 2023
Nanocrystal Surfaces and Defects

October, 17–19, 2023, Rüdesheim, Germany

Scientific Program:
Shelley A. Claridge and Liberato Manna

https://www.beilstein-institut.de/en/symposia/nanocrystals/

Beilstein Organic Chemistry Symposium 2023
π-Conjugated Molecules and Materials

November, 7–9, 2023, Limburg, Germany

Scientific Program:
Chunyan Chi and Aurelio Mateo-Alonso

https://www.beilstein-institut.de/en/symposia/pisystems/

You will find regularly updated information about our symposia at www.beilstein-symposia.org.

Book of Abstracts

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Overview

This symposium will bring together glycochemists and biologists with experts in bioinformatics and computer sciences not only to look back at the roots of glycosciences 30 odd years ago but also to gather the perspectives of present and future applications of glycomics.

Glycomics, in the past considered a subfield of proteomics, has established itself and evolved to become an independent discipline which studies systematically the structures and functions of glycans in a cell or organism.

Consequently, boundaries to proteomics, genomics and metabolomics are crossed towards a systems-wide investigation of the various roles of glycoconjugates in cell-cell communication, recognition processes, signal transduction, molecular trafficking, modulation and regulation of molecular pathways in both eukaryotes and prokaryotes and in many other processes such as diagnosis and therapy of diseases. Glycomics also encompasses further important areas, for example, combining bioinformatics and mathematics approaches to develop methods for data storage, analysis, and modelling functions and relationships.

Under the guidance of the MIRAGE Commission, this conference series also provides a platform to discuss current standards and needs for additional standards in the glycoscience. The mission of MIRAGE (www.beilstein-mirage.org) is to establish guidelines for the reliable and accurate reporting of glycan data and to interconnect existing and future infrastructure entities to provide the glycoscience community services for the deposition, sharing, analysis and reuse of glycan data.

All participants are encouraged to discuss their latest results, approaches, and methodologies in experimental, theoretic and bioinformatics glycosciences.

Enjoy the Symposium!

Scientific Committee

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Registration

All participants must be registered to have access to the conference area.

Participants can ask the organizers for a confirmation of the payment of the conference registration fee. Insurance of participants against accidents, sickness, cancellation, theft, property damage or loss is not covered. Participants are advised to take out adequate personal insurance (see also “Liability and Insurance“).

Participants are responsible for settling their hotel bills directly with the hotel on departure. The total price for participants staying at the Dom Hotel Limburg is 898 EUR and includes both accommodation for four nights and the conference package that covers lunches, dinners and coffee breaks as well as admits access to the conference room.

> **Participants not staying at the Dom Hotel Limburg are requested to register with the hotel for booking and paying the conference package, i.e. 394 EUR per person.**

Extras, such as drinks, telephone calls etc. are not included in the price.

Conference Venue

Both, the conference and lunches and dinners will take place at the conference hotel, i.e.:

Dom Hotel Limburg
Grabenstraße 57
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The hotel offers wireless internet access free of charge. The hotel accepts MasterCard, Visa and EC-Cash (Maestro-Cards).

The Symposium

The symposium will be held from June 20 to 22, 2023, with the 19th and the 23rd for travelling.

The setting and the limited number of participants (max. 60 persons) provide a very convivial atmosphere for the ready exchange of thoughts and ideas.

The scientific program will take place over three days and will

start at 9:00 am on Tuesday, the 20th, and

end in the late afternoon (ca. 5:30 pm) on Thursday, the 22nd.

If you wish to extend your stay, please contact the hotel directly.

For the **length of the individual talks**, please refer to the program. Speakers should allow sufficient time for discussion at the end of their talks (e.g. a 40 min slot includes 30 min talk + 10 min for questions). We will have an LCD projector connected to a Windows PC available.

Presentations of Posters and Software

Poster Exhibition: Tuesday, 20 and Thursday, 22 June, during the coffee breaks

Location of the posters

The poster exhibition will be placed close to the conference room. Your poster board will be marked with your poster number which is same in the abstract book.

Poster mounting

Please mount your poster on Monday, 19th from 6:00 pm or on Tuesday, 20th at the latest by 8:30 am. Your poster will be on display throughout the symposium.

You are asked to remove all poster materials from the board after the coffee break on Thursday, 22nd otherwise it will be taken down on time and disposed by the organizers. The organizers cannot take any responsibility for this material.

Poster material

The size of your poster board is 120 x 90 cm (height x width) and will be marked with your poster number. Hanging material for the poster boards will be provided on-site.

Presentations

The oral poster presentations will take place as indicated in the scientific program. The presentations should not exceed 3 min. You will have 1 min in addition for questions.

Please make sure that you have delivered your final presentation to the organizers in time.

We will have an LCD projector (XGA) connected to a Windows PC available.

Software Demonstration: Tuesday, June 20, and Wednesday, June 21 during the coffee breaks

Location

The software demonstration will take place in a separate room (“Roter Salon”) close to the conference room. Each software presenter will have a separate table marked with your software demo number which will remain the same in the abstract book.

Material

Please bring in your own device for the software demo. Please make sure in time that everything is running and login the wireless LAN for internet if necessary.

Presentations

The elevator pitch presentations will take place as indicated in the scientific program. The presentations should not exceed 3 min. You will have 1 min in addition for questions.

Please make sure that you have delivered your final presentation to the organizers in time.

We will have an LCD projector (XGA) connected to a Windows PC available.

Liability and Insurance

The Beilstein-Institut will not be liable for any accident, theft or damage to property, nor for any delays or modification in the program due to unforeseen circumstances.

Participants and accompanying persons are advised to arrange personal travel and health insurance.

Scientific Program

Monday, June 19

19.30 Welcome reception

20.00 Dinner

Tuesday, June 20

09:00	Opening and Introductory Remarks	Carsten Kettner
	Session Chair: Benjamin Schumann	
09:20	The Diversity and Functionality of the Cancer Glycome: From Molecular Mechanisms to the Clinical Applications	Celso A. Reis
10:00	Glycoproteomic Landscape and Structural Dynamics of TiM Family Immune Checkpoints Enabled by Mucinase SmE	Stacy A. Malaker
10:40	Software Lightning Talks *Chaired by René Ranzinger*	
11:00	*Coffee Break and Software Demo*	
11:40	Regulation of N-linked Glycosylation Site Occupancy	Benjamin L. Schulz
12:20	Autonomous Optimization of Glycosylations Generates Reliable Data for Machine Learning Approaches to Reaction Prediction	Peter H. Seeberger
13:00	*Lunch*	
	Session Chair: Elisa Fadda	
14:15	Information Transfer in Mammalian Glycan-based Communication	Christoph Rademacher
14:55	Map of Neuronal Tissue O-glycosites Reveal Specific Traits in the Secretogranin Family	Katrine Schjoldager
15:35	Poster Lightning Talks #1 Posters 1-8	
16:05	*Conference Photo, Tea Break and Poster Session*	
16:35	Poster Lightning Talks #2 Poster 9-17	
17:05	Synthesis and Function of Capsular Modified Heptoses in *Campylobacter jejuni*	Carole Creuzenet

17:45	Chemical Evolution of Enzyme-catalyzed Glycosylation	Hongzhi Cao
18:25	*Close*	
19:30	*Dinner*	

Wednesday, June 21

	Session Chair: Carole Creuzenet	
09:00	*Opening*	
09:05	Mucin-derived Glycans as Regulators of Pathogen Virulence	Rachel Hevey
09:45	S-Layer Glycosylation Patterns of Tannerella Species in Oral Health versus Disease Effectors and Effects	Christina R. Schäffer
10:25	*Coffee Break , Poster Session and Software Demo*	
11:00	Structural Insights into the Function of *Toxoplasma gondii* Mucin-type O-glycosyltransferase GalNAc-T3	Nadine L. Samara
11:40	Measuring Glycoprotein Host-pathogen Interactions by Single-molecule Mass Imaging and Tracking	Weston Struwe
12:20	Chemical Precision Tools Reveal the Surprising Biology of SARS-CoV-2 O-glycosylation	Benjamin Schumann
13:00	*Lunch*	
14:00	*Excursion*	
19:30	*Dinner*	

Thursday, June 22

09:00	*Opening*	
	Session Chair: Niclas Karlsson	
09:05	The Diversity of the Glycan Shield of Sarbecoviruses Related to SARS-CoV-2	Joel D. Allen
09:45	Sugars as Key Elements of Archetypical Biopolymers Motifs: The Case of C-Mannosylation of Thrombospondin Repeats	Elisa Fadda
10:25	*Coffee Break and Poster Session*	
11:10	Making Glycodata as FAIR as Possible	Frédérique Lisacek
11:50	The Glycan Array Data Repository	René Ranzinger
12:30	New Native Mass Spectrometry Tools for Glycomics	John S. Klassen
13:10	*Lunch*	
	Session Chair: Daniel Ungar	
14:30	Full-stack Glycoinformatics: From Data to Insight	Daniel Bojar
15:10	Exploring Functional Impact of Single Nucleotide Variations through Integration of Genomic and Glycosylation Site Specific Data in GlyGen	Raja Mazumder
15:50	*Coffee Break*	
16:20	Applying Transcriptomics to Study Glycosylation at the Cell Type Level	Hiren J. Joshi
17:00	Studying the Mechanism of Bacterial Protein Glycosylation	Marthe T. C. Walvoort
17:40	Elucidating Bacteria Toxin and Mucin Binding Domains – Specificities Towards Mucin-O-Glycopeptides	Ulrika Westerlind
18:20	*Closing Remarks*	Carsten Kettner
19:30	*Dinner*	

List of Posters

The poster presentation includes a short (3 min) oral presentation on Tuesday, June 20, and the poster sessions during the coffee breaks on Tuesday and Thursday. The posters will be displayed throughout the entire symposium from Tuesday, June 20, to Thursday, June 22.

#1	Exploring Protein *N*-glycosylation Alterations between Wild-type and Mutant Forms of Irisin	Joe Zaia
#2	Towards the Development of a Spatio-temporal O-Glycosylation Reporter System	Abdul H. Zafar
#3	Modelling the Processing of Glycans on Secreted Glycoproteins	Daniel Ungar
#4	Mapping Glycosyltransferases Glycosylation Sites and Substrate Preferences via Glycoproteomics	Lucia Di Vagno
#5	Evolution of the Interactions between Rotavirus VP8* and HBGAs: Insights from Computational Studies	Jesus Rodriguez-Diaz
#6	Fine-tuning the Spike: Role of the Glycan Shield in the Structure/Dynamics of SARS-CoV-2S	Carl Fogarty
#7	Simple, yet Logical Terms of N-glycans Meet the Emergence of Isomer-sensible Analysis	Friedrich Altmann
#8	Towards Clinical O-glycomics: Applications for Osteoarthrithis	Niclas Karlsson
#9	Exploring the Glyco/Proteome of the Australian Sheep Blowfly for Better Vaccine Targets	Edward Kerr
#10	*Elucidating the O-glycan Structure of Tannerella serpentiformis*	Stephanie Walcher
#11	Wholistic Heparan Sulphate oligosaccharide Profiling using Top-down GAGomics	Marisa L. Maciej-Hulme
#12	Development of a Machine Learning Framework to Extract the Biomarker Potential of IgG *N*-glycans	Konstaninos Flevaris
#13	Serum *N*-glycan Biomarkers Predict Patient Response to Vedolizumab Treatment for Crohn's Disease	Georgia Elgood Hunt

#14	Integrated Glycomics and Genomics Reveals a Role for Plasma Protein and IgG *N*-glycosylation in Type 1 Diabetes Development	Nadja Rudman
#15	Linking Glycosylation to Function	Catherine Hayes
#16	Bioinformatics Analyses of Nematode Hexosaminidases	Zuzanna Dutkiewicz
#17	A Bacterial Mannose Binding Lectin as a Tool for the Enrichment of C-and O- Mannosylated Peptides	Hans Bakker

List of Software Demonstrations

The software demonstrations includes a short (3 min) oral presentation on Tuesday, June 20, as well as the demo sessions during the coffee breaks as indicated in the program.

#1	Privateer – Validation of Modelled Glycosylation in the 3D Structural Biology Frontier	Haroldas Bagdonas
#2	Automated Glycopeptide Data Processing and Curation in High Sensitivity Applications	David Falck
#3	A Demonstration of CarbArrayART: a Tool for Glycan Microarray Data Management and Reporting	Yukie Akune
#4	What's New on Glyco@Expasy?	Frédérique Lisacek
#5	GlycoDomainViewer, Glycopacity and Glyco.me	Hiren Joshi
#6	GlyFinder: An Online Tool for Finding Glycans and Glycoproteins in the PDB	Robert Woods
#7	GlyGen portal, glycan array data repository and the Glycoinformatics Consortium	René Ranzinger

Abstracts

Tuesday

09:20

The Diversity and Functionality of the Cancer Glycome: From Molecular Mechanisms to the Clinical Applications

Celso A. Reis

University of Porto
Institute for Research and Innovation in Health
Porto, Portugal

Glycans are key components of biological systems underlying a variety of essential structural and functional roles. Glycans control and define fundamental molecular, cellular, tissue, organ and systemic biological processes directing physiological functions and being involve in several human diseases, such as cancer [1,2].

Cancer is a heterogeneous and complex disease that requires the understanding of the different components underlying the biology of the tumour. Alterations of glycosylation are common molecular alterations during carcinogenesis [3] with major biological implications for cancer progression [1,2]. In this presentation the basis underlying some of the most common alterations of glycosylation that occur in cancer will be reported, such as those leading to the alteration of the cancer cell glycoproteome and the aberrant expression of the truncated O-glycans [4,5]. In addition, this presentation will highlight recent glycomic and glycoproteomic strategies that provided crucial information on the alterations of glycosylation that impact: (a) the activation of oncogenic receptors [6-10] such as the HER2 and EGFR that display site-specific glycosylation profiles affecting the biology of the receptor and the sensitivity of cancer cells to therapeutic humanized monoclonal antibodies used in the clinics [8,9]; (b) the role of glycans in cancer cell extracellular vesicle communication [11]; and (c) the function glycans in the tumour microenvironment, immune response and immunotherapy [1,2,3].

The presentation is going to highlight the functional aspects of glycosylation modifications occurring in cancer and their potential application as biomarkers for patient stratification, personalize medicine and for novel and improved therapeutic applications [1,8,9].

References

[1] Mereiter S. *et al.*, *Cancer Cell*. 2019;36(1):6-16.

[2] Pinho S, Reis CA. *Nature Rev. Cancer* 2015, 15, 540-555.

[3] Magalhães *et al.*, *Biochim Biophys Acta*. 2015;1852(9):1928-39.

[4] Marcos N *et al.*, *Cancer Res.* 2004; 64(19):7050-7.
[5] Campos *et al.*, *Mol Cell Proteomics*. 2015;14(6):1616-29.
[6] Gomes C. *et al.*, *PLoS One*. 2013; 8(6):e66737.
[7] Mereiter S. *et al.*, *Biochim Biophys Acta*. 2016;1860(8):1795-808.
[8] Duarte HO. *et al.*, *Oncogene*. 2021;40(21):3719-3733.
[9] Rodrigues J. *et al. Cell Oncol*. 2021; 44(4):835-850.
[10] Freitas D. *et al.*, *EBioMedicine*. 2019; 40:349-362.
[11] Freitas D. *et al.*, *J Extracell Vesicles*. 2019;8(1):1621131.

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Tuesday

10:00

Glycoproteomic Landscape and Structural Dynamics of TiM Family Immune Checkpoints Enabled by Mucinase SmE

Stacy A. Malaker

Yale University
Department of Chemistry
New Haven, CT, United States of America

Mucin-domain glycoproteins are densely *O*-glycosylated and play critical roles in a host of biological functions. In particular, the T cell immunoglobulin and mucin-domain containing family of proteins (TIM-1, −3, −4) decorate immune cells and act as key checkpoint inhibitors in cancer. However, their dense O-glycosylation remains enigmatic both in terms of glycoproteomic landscape and structural dynamics, primarily due to the challenges associated with studying mucin domains. Here, we present a mucinase (SmE) and demonstrate its ability to selectively cleave along the mucin glycoprotein backbone, similar to others of its kind. Unlike other mucinases, though, SmE harbors the unique ability to cleave at residues bearing extremely complex glycans which enabled improved mass spectrometric analysis of several mucins, including the entire TIM family.

With this information in-hand, we performed molecular dynamics (MD) simulations of TIM-3 and −4 to demonstrate how glycosylation affects structural features of these proteins. Overall, we present a powerful workflow to better understand the detailed molecular structures of the mucinome.

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11:40

Regulation of *N*-linked Glycosylation Site Occupancy

Benjamin L. Schulz

The University of Queensland
School of Chemistry and Molecular Biosciences
Brisbane, Australia

The key post-translational modification process of protein glycosylation helps proteins fold, regulates the functions of mature glycoproteins, and is important in essentially all biological processes involving cell-cell or protein-cell interactions. As a key cellular process affecting most secreted and membrane proteins, perturbations to the normal regulation of glycosylation can affect diverse proteins. To study the physiological regulation of protein glycosylation we have used Data-Independent Acquisition Liquid Chromatography Mass Spectrometry (DIA LC-MS/MS) in combination with yeast genetics.

We have identified and characterised how oligosaccharyltransferase, the central enzyme in the process of *N*-glycosylation, has extensive flexibility in selecting glycan donor and protein acceptor substrates, and how this balance of flexibility and selectivity contributes to regulation of site-specific glycosylation at a systems level.

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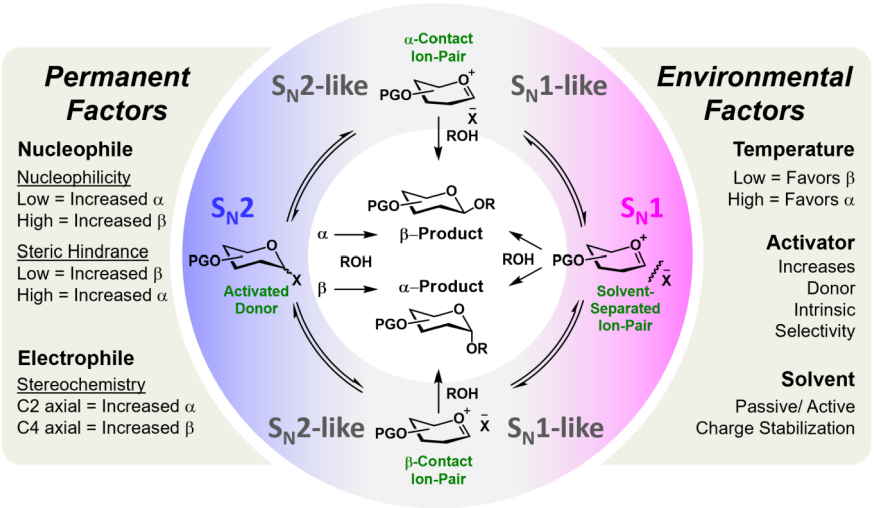
12:20

Autonomous Optimization of Glycosylations Generates Reliable Data for Machine Learning Approaches to Reaction Prediction

Peter H. Seeberger

Max Planck Institute of Colloids and Interfaces
Biomolecular Systems Department
Potsdam, Germany

Predicting the stereochemical outcome of chemical reactions is challenging in mechanistically ambiguous transformations. Glycosylation is one of the most mechanistically complex organic transformations, where an electrophile (donor), upon activation with a Lewis or Brønsted-Lowry Acid, is coupled to a nucleophile (acceptor) to form a C-O bond and a stereogenic center. This reaction involves numerous potential transient cationic intermediates and conformations and can proceed via mechanistic pathways spanning S_N1 to S_N2. The stereochemical outcome is determined by more than eleven permanent (defined by the starting materials) or environmental factors (defined by the selected conditions/catalyst) whose degree of influence, interdepency, and relevance is poorly understood.



Using an autonomous, continuous flow set-up empirical evidence was obtained to a highly reproducible, concise dataset as basis to train a random forest algorithm to accurately predict the stereoselective outcome of glycosylations.

The steric and electronic contributions of all chemical reagents and solvents were quantified by quantum mechanical calculations. The trained model accurately predicts stereoselectivities for unseen nucleophiles, electrophiles, acid catalyst, and solvents across a wide temperature range (overall root mean square error 6.8%). All predictions were validated experimentally on a standardized microreactor platform. The model helped to identify novel ways to control glycosylation stereoselectivity and accurately predicts previously unknown means of stereocontrol.

References

[1] Chatterjee, S.; Moon, S.; Hentschel, F.; Gilmore, K.; Seeberger, P.H.; An Empirical Understanding of the Glycosylation Reaction; *J. Am. Chem. Soc.,* **2018**, *140*, 11942-11953.
[2] Moon, S.; Chatterjee, S.; Seeberger, P.H.; Gilmore, K.; Predicting Glycosylation Stereoselectivity Using Machine Learning; *Chem. Sci.* **2021**, *12,* 2931-2939.

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Tuesday

14:15

Information Transfer in Mammalian Glycan-based Communication

Christoph Rademacher

University of Vienna
Department of Pharmaceutical Sciences
Vienna, Austria

Surface exposed lectins on mammalian cells decipher the information encoded within glycans exposed on an external input such as neighboring cells or pathogens. This recognition leads to the translation of the incoming glycan-based signal into a biochemical signal in the cell. Here we will present approach based on information theory to study glycan-lectin communication pathways using single-cell-resolved quantitative datasets. For this, we chose C-type lectin receptors expressed on immune cells as a model system. NF-κB-reporter cell lines expressing DC-SIGN, MCL, dectin-1, dectin-2, and mincle were used on the background of a U937 cells and compared their transmission of glycan-encoded information. Using the framework, we found how multiple lectin receptors are utilized by the immune cells to integrate incoming information and whether the signaling capacity of lectin receptors using similar signal transduction pathways are being compromised or enhanced. Our approach provides a new framework to understand how weak multivalent interactions can be analyzed and understood as a formal communication channel.

Reference

Felix F Fuchsberger, Dongyoon Kim, Natalia Baranova, Hanka Vrban, Marten Kagelmacher, Robert Wawrzinek, Christoph Rademacher (2023) Information transfer in mammalian glycan-based communication. *eLife* 12:e69415. https://doi.org/10.7554/eLife.69415.

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Tuesday

14:55

Map of Neuronal Tissue O-glycosites Reveal Specific Traits in the Secretogranin Family

Madsen, T.D.[1], Topaktas, A.B.[1], Dworkin, L.A.[1], Hintze, J.[1], Hansen, L.H.[1], Nikpour, M.[2], Miller, R.[1], Vakhrushev, S.Y.[1], Joshi, H.J.[1], Katrine Schjoldager[1]

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University of Gothenburg
Department of Laboratory Medicine
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Protein O-glycosylation is a major post translational modification of proteins. When O-glycosylation is impaired severe diseases may develop, most with clear metabolic and neurological involvement. However, the understanding of molecular mechanisms involving glycoproteins in neuronal development and metabolic signaling has been halted by technical limitations. Now, we mapped O-GalNAc- type glycosylation sites in neuronal and endocrine glycoproteins by performing targeted glycoproteomics on multiple biosources. Surveying the resulting >7.000 O-GalNAc-type glycosites in >3.000 proteins, we identified multiple glycosylation sites within major protein classes involved in biological niches such as perineural nets, synapse formation, axon guidance, endocytosis and granulogenesis.

Here, we pursued the functions of the newly identified glycosylation sites in one of the identified families, the secretogranins, that are key players in neurotransmitter and hormone signaling. To this end we have taken a genetic engineering approach where selective ablation of key glycosyltransferase genes enables removal of single types of protein glycosylation pathways, while keeping the remainder of the glycome intact. Collectively our data provide a road map of neuronal protein O-glycosylation for further studies in neuroendocrine research.

As an example, we generate a glycosite submap on the pleiotropic and granulogenic Secretogranin family members. We show that the key member, Chromogranin A, contain high but subsaturated stoichiometry of O-glycans and that this glycosylation is important for proper multimerization.

In line with these results, neuronal cell lines deficient in O-glycosylation show higher capacity for the neurotransmitter noradrenaline and expanded neurotransmitter-containing dense core granules.

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Tuesday

17:05

Synthesis and Function of Capsular Modified Heptoses in *Campylobacter jejuni*

Carole Creuzenet[1], M. Myles[1], B. Yang[1], H. Barnawi[1], N. Fava[1], L. Woodward[2], D. Zimmerman[1], and J. Naismith[2]

[1]University of Western Ontario, Department of Microbiology and Immunology, London, Ontario, Canada
[2]Oxford University, Division of Structural Biology, Oxford, United Kingdom

The bacterium *Campylobacter jejuni* (CJ) is a common cause of gastroenteritis in humans, with most transmission in developed countries resulting from the ingestion of contaminated broiler chicken meat. CJ resides commensally in the gastrointestinal tract of this avian reservoir, with disparities in host commensalism (in poultry) vs pathogenicity (in humans) potentially resulting from differential innate immune activation. A key mediator of interactions with host cells which impacts both colonization and virulence is the external capsular polysaccharide. The capsule comprises uniquely modified heptoses whose synthesis and function was unknown. We investigate their biosynthesis and biological role with a view to inhibit their formation and reduce the incidence of campylobacteriosis.

Our knockout mutagenesis studies of heptose modifying genes in strain NCTC 11168 where the modified heptose branches off the capsular backbone, showed that heptose modification is not necessary for capsule synthesis but is essential for capsular function as a virulence factor. Namely, it affects bacterial resistance to serum and bile salts, biofilm formation, and adhesion to human intestinal epithelial cells and their invasion. The mutants also showed altered interactions with macrophages (adhesion, uptake and intracellular survival, activation patterns), with species-specific effects across human and avian macrophages. We also demonstrate that heptose modifying genes are important for colonization and persistence of *C. jejuni* in chicken. These findings suggest that fine tuning the capsule composition via heptose modification contributes to host pathogen interactions and likely to host specificity.

We also elucidated the enzymatic pathways for heptose synthesis in 2 CJ strains that produce slightly different heptoses and identified conserved C3, C5 epimerases and C4 reductases that could be targeted for inactivation.

We determined their role in catalysis and in substrate and product specificity using site directed mutagenesis and capillary electrophoresis. Key catalytic residues were identified, some differing from predictions based on well-studied hexose modifying epimerases and reductases. Several residues that govern C3 vs C5 heptose epimerization specificity and others that govern heptose vs hexose substrate specificity were identified, which is important to enable development of inhibitors specific for heptose modifying enzymes. Also, we determined the structure of 4 of these enzymes with and without substrate. Overall, our data explains how highly similar enzymes perform different epimerization reactions. Finally, preliminary data identified 2 candidate inhibitors for the epimerases. Their characterization is underway.

This work provides new enzymes that can be targeted for inhibition to decrease human campylobacteriosis by application of inhibitors to chickens pre-slaughter to reduce chicken colonization, thus decreasing meat contamination by CJ during the slaughter process and reducing the risk of downstream human campylobacteriosis. The identified features of the substrate binding site and its specificity can be used to tailor our candidate inhibitors to increase their efficacy. This work also provides new tools to synthesize carbohydrate antigens useful for chicken vaccination and provides grounds for the elucidation of similar pathways of other pathogens.

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Chemical Evolution of Enzyme-catalyzed Glycosylation

Hongzhi Cao

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Shandong, China

Despite tremendous advances in chemical glycosylation in the past few decades, the synthesis of biologically important complex carbohydrates in appreciable amounts remains challenging. An attractive alternative to chemical synthesis is enzymatic synthesis, which proceeds in high regio- and stereoselectivity without tedious protecting group manipulation. However, the enzymatic synthesis of complex carbohydrate is also challenging as it involves an array of carbohydrate processing enzymes, especially different enzyme isoforms. To overcome this limitation, the diversity-oriented modular enzymatic assembly strategy has been developed for the rapid assembly of various complex glycans in a few steps. In this modular system, each module comprises a group of bacterial enzymes for *in situ* generating the corresponding sugar nucleotide donor and a bacterial glycosyltransferase responsible for transferring the monosaccharide from sugar nucleotide to an acceptor in one-pot. Several chemical concepts were successfully employed to this modular system to control the regioselective and vary the target glycan sequence.

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09:05

Mucin-derived Glycans as Regulators of Pathogen Virulence

Rachel Hevey

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Basel, Switzerland

The mucosal barrier is well-established to play an important role in microbiota development and as a first line of host defense. Although this has traditionally been attributed to the physicochemical properties of mucus, recent reports indicate that mucin glycoproteins and their associated glycans can regulate gene expression and are capable of attenuating virulence in diverse, cross-kingdom pathogens, including Gram-positive bacteria, Gram-negative bacteria, and fungi.

With mucins displaying several hundred distinct glycan structures, we sought to identify discrete glycan structures responsible for this novel gene regulation. Individual mucin O-glycan structures are not commercially available, are not yet amenable to automated synthesis, and given their overlapping physical and chemical properties cannot be isolated as pure compounds from natural sources using current technologies.

Therefore, through a multi-centre collaborative effort (full list of contributors in [1-3]) we have been actively: (i) characterizing complex mucin O-glycan pools to identify structures most likely to display biological activity; (ii) developing a synthetic approach to obtain individual mucin O-glycans in sufficient quantity for functional analysis [2]; and (iii) assessing the virulence attenuating capabilities of individual glycans in diverse pathogens [1,3]. Within this framework, we have successfully identified specific structures that suppress virulence phenotypes in the fungal pathogen *Candida albicans* (e.g., filamentation, biofilm formation), and regulate pathogenicity in *Vibrio cholerae* (e.g., reduced cholera toxin production), with potency comparable to native mucin glycan pools.

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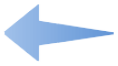
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S-Layer Glycosylation Patterns of *Tannerella* Species in Oral Health *versus* Disease Effectors and Effects

Christina R. Schäffer

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The cell surface of the oral biofilm pathogen *Tannerella forsythia* is heavily glycosylated with a complex decasaccharide that is O-glycosidically linked to a phylum-wide protein glycosylation motif of the bacterium's abundant surface (S-) layer [1] as well as other proteins [2]. The S-layer glycoproteins are virulence factors of *T. forsythia;* they are exported via a type 9 secretion system [3,4] and there is evidence that protein O-glycosylation underpins bacterial pathogenicity [5], critically involving a strain-specific nonulosonic acid [6-8].

O-Glycosylation pathway genes are encoded in a polycistronic, genomic gene cluster. Using a gene deletion approach targeted at predicted glycosyltransferases [9] and methyl-transferases encoded in this gene cluster, in combination with mass spectrometry of the protein-released O-glycans, we showed that the gene cluster encodes the species-specific part of the decasaccharide and that this is assembled step-wise on a pentasaccharide core [10]. The O-glycan is pivotal to the positioning of *T. forsythia* within a multispecies oral model biofilm [11] and to the modulation of DC effector functions, with the *T. forsythia*-specific glycan portion suppressing and the pentasaccharide core activating a Th17 response [10]. Notably, all sequenced genomes of *T. forsythia* strains reveal glycosylation gene clusters of similar size and organization. In contrast, the corresponding region in the genome of the periodontal health-associated bacterium *Tannerella serpentiformis*, which is the closest phylogenetic relative of *T. forsythia*, shows a different gene composition lacking nonulosonic acid biosynthesis genes.

Our data support the potential of *T. serpentiformis* to interfere with biological processes relevant to the establishment of periodontitis, evidenced, for instance, by a decreased invasiveness of human gingival fibroblasts and macrophages compared to *T. forsythia* as well as the notable capability to outcompete pathogenic *T. forsythia* in a periodontitis biofilm model [12]. Ongoing structure elucidation of the *T. serpentiformis O*-glycan confirms the presence of a novel O-glycan devoid of nonulosonic acids. Thus, we hypothesize that nonulosonic acid O-glycans are a hallmark of pathogenic *T. forsythia* strains. This opens up an avenue for *O*-glycosylation engineering as a potential strategy to interfere with the establishment of periodontitis.

This work was supported by the Austrian Science Fund FWF, projects P 20605, P 24317, P 33618, P34642 and W1224.

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11:00

Structural Insights into the Function of *Toxoplasma gondii* Mucin-type O-glycosyltransferase GalNAc-T3

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Mucin-type O-glycosylation is a post-translational modification that results in the addition of O-glycans to Ser or Thr residues on proteins and is conserved across eukaryotes, including *Toxoplasma gondii (T. gondii)*, an obligate intracellular protozoan parasite that infects one-third of the world's population and causes toxoplasmosis. Host infection by *T. gondii* is associated with the formation of latent cysts in the central nervous system that are enveloped with a structure called the cyst wall, whose rigidity is imparted by the O-glycosylation of the mucin-like domain of the cyst wall proteins such as CST1. *T. gondii* contains 5 members of a conserved family of enzymes termed N-acetylgalactosaminyltransferases (GalNAc-Ts), which initiate mucin-type O-glycosylation: *T. gondii*GalNAc-T1 to T5. *T. gondii*GalNAcT2 is required for the initial glycosylation of the mucin-like domain of CST1, and neighboring acceptor sites are then O-glycosylated by the glycopeptide preferring isoenzymes *T. gondii*GalNAcT-T1 and T3. Previous studies showed that *T. gondii*GalNAc-T2 and -T3 deletion mutants produce various O-glycosylation defects on the cyst wall and reduce the infectivity of the parasite. Reduced sequence homology between *T. gondii* and metazoan GalNAc-Ts hints that an evolutionary divergence may have occurred in substrate recognition and enzyme function, suggesting that *T. gondii*GalNAc-T2 and T3 may be specifically inhibited and are thus potential drug targets for toxoplasmosis. X-ray crystal structures of *T. gondii*GalNAc-T3 in complex with glycopeptides from 2.5-2.9 Å resolution show that *T. gondii*GalNAc-T3 substrate specificity is dictated by a unique GalNAc binding pocket that recognizes an existing GalNAc on a substrate one amino acid C-terminal to the acceptor site.

The structures also reveal a 2nd novel metal binding site that appears to help align the GalNAc binding pocket, and mutations of residues involved in both GalNAc and 2nd metal binding reduce enzymatic activity in vitro. In the future, we hope to understand how the unique GalNAc pocket and 2nd metal site residues affect *T. gondii*GalNAc-T3 function *in vivo* to further build a foundation for understanding and targeting mucin-type O-glycosylation in cyst wall formation and *T. gondii* pathogenesis.

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Measuring Glycoprotein Host-pathogen Interactions by Single-molecule Mass Imaging and Tracking

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Oxford, United Kingdom

Enveloped virus spikes are highly complex glycoprotein assemblies that are metastable, dynamic and heavily glycosylated – each of these features contribute to how viruses interact within the host. Glycosylation of enveloped spikes, particularly HIV-1 Env, is fundamental for efficacy of structure-based vaccines as many broadly neutralising antibodies (bnAbs) incorporate glycans as part of their binding epitopes. Although immune evasion has been attributed to changes in glycosylation, due to high rates of mutation on Env, an additional principle is based on the surface structure of individual HIV-1 virions. Here, low Env density and geometric constraints limit intra-spike crosslinking and epitope binding by neutralising antibodies which leads to poor immune responses. Our work explores these principles and aims to understand the mechanisms by which bNAbs and receptors interact with viral glycoproteins on membrane surfaces. Our methodological approach involves various mass spectrometry methods (glycomics/glycoproteomics, hydrogen-deuterium exchange and native MS) as well as single molecule mass imaging and tracking by mass photometry.

This new method, termed dynamic MP, allows us to quantify diffusion and binding of bnAb-Env assemblies on supported lipid bilayers. Overall, these data help shed light on HIV-1 immune evasion across molecular scales, from molecular detail of glycosylation to biophysical restraints involved in diffusion and antibody-mediated crosslinking of viral spikes.

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Chemical Precision Tools Reveal the Surprising Biology of SARS-CoV-2 Glycosylation

Edgar Gonzalez-Rodriguez[1,2], Mia Zol-Hanlon[1,2], Ganka Bineva-Todd[1,2], Andrea Marchesi[1,2], Lucia Di Vagno[2], Benjamin Schumann[1,2]

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O-GalNAc glycans are found on the majority of proteins trafficking through the secretory pathway, including viral glycoproteins [1]. Despite their profound impact on physiology, studying O-GalNAc glycans is challenging due to their biosynthetic complexity: As many as 20 different glycosyltransferase isoenzymes of the GalNAc-T family prime glycosylation with GalNAc on Ser/Thr residues. Downstream elaboration includes glycosylation with other monosaccharides such as Gal and Sia. In a chemical biology campaign, we recently developed a tactic termed bump-and-hole engineering to dissect the glycosylation sites introduced by individual GalNAc-T isoenzymes [2-3]. The tactic combines enzyme engineering with the use of bioorthogonal ("clickable") monosaccharides to provide unique insight into the biology of O-GalNAc glycans.

The SARS-CoV-2 Spike protein has become one of the most-studied glycoproteins in contemporary biomedicine. Maturation of Spike includes proteolytic maturation in the secretory pathway by a number of proteases. Despite a wealth of information on *N*-glycosylation of Spike [5,6], the position and roles of O-glycans have been understudied, mainly due to a lack of relevant tools. Ten Hagen and colleagues revealed an association between O-GalNAc glycosylation and proteolytic maturation, as well as downregulation of O-glycosylation in Spike proteins of variants of concern [7]. Complementing these studies, we employed a host of chemical tools to unravel surprising details of O-glycan-mediated impact on the maturation of Spike [8]. Through bump-and-hole engineering and chemical methods to enhance glycoproteome analysis, we found that the enzyme GalNAc-T1 glycosylates Ser678 in the secretory pathway of human cells.

Suitable reporter probes for proteolytic cleavage then revealed that GalNAc elaboration with Sia substantially impairs furin cleavage. Variants of concern including Alpha, Delta and Omicron carry mutations to avoid glycosylation by GalNAc-T1, in evolutionary trajectories that may by independent from inherently increasing proteolysis. These findings posit O-glycans as an important driver of the evolution in variants of concern, highlighting the role of these glycans in viral transmission

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Thursday

09:05

The Diversity of the Glycan Shield of Sarbecoviruses Related to SARS-CoV-2

Joel D. Allen

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School of Biological Sciences, Molecular and Cellular Biosciences
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Animal reservoirs of sarbecoviruses represent a significant risk of emergent pandemics, as evidenced by the SARS-CoV-2 pandemic. Vaccines remain successful at limiting severe disease and death, however the potential for further coronavirus zoonosis motivates the search for pan-coronavirus vaccines. This necessitates a better understanding of the glycan shields of coronaviruses, which can occlude potential antibody epitopes on spike glycoproteins. Here, we compare the structure of 12 sarbecovirus glycan shields. Of the 22 *N*-linked glycan attachment sites present on SARS-CoV-2, 15 are shared by all 12 sarbecoviruses. However, there are significant differences in the processing state at glycan sites in the N-terminal domain, such as N165. Conversely, glycosylation sites in the S2 domain are highly conserved and contain a low abundance of oligomannose-type glycans, suggesting a low glycan shield density. The S2 may, therefore, provide a more attractive target for immunogen design efforts aiming to generate a pan-coronavirus antibody response.

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Sugars as Key Elements of Archetypical Biopolymers Motifs: The Case of C-Mannosylation of Thrombospondin Repeats

Elisa Fadda

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The functionalization of proteins with complex carbohydrates plays multiple and fundamental roles in the biology of health and disease. One of the most important and yet underestimated functions of glycosylation is directly related to regulation of protein folding. In this talk I will present and discuss the results of recent work in collaboration with A/Prof Ethan Goddard Borger's team at WEHI, Melbourne, showing how a rare, yet highly specific type of glycosylation, namely C-mannosylation (C-Man), modulates the folding and regulates the stability and function of thrombospondin repeats (TSR) in the N-terminal domain of Brain-specific Angiogenesis Inhibitor 1 (BAI-1) adhesion GPCRs.

Based on these findings I will introduce further evidence supporting the overarching role of sugars as keystones determining the 3D structure of biopolymers.

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Making Glycodata as FAIR as Possible

Frédérique Lisacek

Swiss Institute of Bioinformatics
Proteome Informatics Group
Geneva, Switzerland

With the increase in publications dedicated to the solving of carbohydrate structures there is a need for well curated, annotated and searchable databases to disseminate this work.

Several groups now work in fulfilling this role, and among them, Glyco@Expasy (glycoproteome.expasy.org) GlyCosmos (www.glycosomos.org) and GlyGen (www.glygen.org) have come together to create the GlySpace Alliance (www.glyspace.org), a cross-country/continent alliance to aid the glycobiology community by sharing and collaborating on glycobioinformatic resources on a FAIR basis. Each resource brings its own expertise to the collaboration with global or pairwise initiatives. A key aspect to unifying resources is to make the journey between a glycoprotein and a glycan-binding protein/lectin transparent and easily accessible. This talk will highlight how this way can be paved.

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The Glycan Array Data Repository

René Ranzinger[1], Sena Arpinar[1], Sujeet Kulkarni[1], Raja Mazumder[2], Mike Tiemeyer[1]

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Glycan arrays have grown greatly over the past 2.5 decades regarding the diversity of the immobilized glycans they present on the slides for query of binding activities and the broad range of biomedical domains they have impacted. The rich datasets generated by various internationally developed array formats have provided new knowledge regarding the structural specificities of known glycan binding proteins and new discoveries of previously unexpected glycan binding activities. These binding activities, when integrated with knowledge of the tissue, cell, and disease-specific expression of the candidate binding proteins and their recognized glycan ligands, promise to further accelerate appreciation of glycan structure and function in disease and normal tissue.

Although, a huge number of array datasets have been generated since the first glycan arrays became available around 2000, a resource that systematically archives glycan array data and associated metadata is lacking. A few groups or consortia, such as the CFG and the Glycosciences Laboratory (Imperial College London), provide their own data on their respective group webpages. However, these webpages do not allow for public submission of data and are limited to the data generated by the corresponding research group. Furthermore, there has been no agreement on metadata or common data formats between these groups that would enhance the interoperability and FAIRness of the data. The Glycan Array Data Repository is a public database which allows submission of array data independent of the array format or investigator-specific features.

The submission system supports multiple glycan array raw data formats and requests associated metadata in accordance with the glycan array minimum information guidelines developed by the MIRAGE initiative (https://www.beilstein-institut.de/en/projects/mirage/).

Uploaded data and metadata are harmonized into common data formats and stored in the database together with the originally submitted files. Once data is released to the public, datasets can be searched, browsed, and downloaded from the repository webpages without the need of a user account.

The Glycan Array Data Repository is support by NIH Glycoscience Common Fund (1U01GM125267-01).

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New Native Mass Spectrometry Tools for Glycomics

John S. Klassen

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Native mass spectrometry (nMS) – electrospray ionization (ESI)-MS carried out under physiological solution conditions with experimental/instrumental parameters that preserve the non-covalent interactions present in solution – has become an indispensable tool in glycomics research. When performed with the catch-and-release (CaR)-ESI-MS technique, nMS serves a sensitive, label-free method for screening glycan libraries (defined and natural) against glycan-binding proteins (GBPs) and accelerates the discovery of ligands and provides new insights into the fine glycan specificity of endogenous and exogenous GBPs. When combined with model membranes, such as nanodiscs, nMS allows for the detection of glycosphingolipid ligands and the quantification of their interactions with GBPs. Implementation of nMS in a time-resolved manner enables the precise measurement of the kinetics and substrate specificities of carbohydrate-active enzymes (CAZymes).

This talk will review recent advances in nMS methods for natural glycan library screening (concentration-independent (COIN) nMS) and quantification of glycan/glycoconjugate interactions with GBPs (slow-mixing mode (SLOMO) nMS). New methods capable of quantifying the relative substrate specificities of CAZymes will also be presented, together with examples of how this information can be exploited for precision glycoengineering and cancer diagnostic applications.

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Full-stack Glycoinformatics: From Data to Insight

Daniel Bojar

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Data processing and analysis in glycobiology can often be viewed as islands: isolated, heterogeneous, and personalized. This creates a high barrier of entry, decreases scale by semi-manual processes, impacts reproducibility, and usually does not generalize to a different aspect of a field as diverse as glycobiology.

We present a complete and generalizable workflow, starting from glycomics mass spectrometry raw files and ending in visualized glycan structures, their biosynthetic relationships, motif enrichment or dysregulation, functional predictions via AI models, and more. All this is scalable to big-data and fully implemented in Python and open-access, orchestrated by our core platform glycowork, and presented in high-level wrapper functions with smart defaults that allow users with minimal coding experience to engage with these methods, lowering the threshold for state-of-the-art analysis substantially.

We envision that this unification of established and new modules for glycoinformatics will yield improvements for every step of glycan data processing and analysis, leading to an acceleration of AI-enhanced glycobiology and new insights into the roles of glycans.

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Exploring Functional Impact of Single Nucleotide Variations through Integration of Genomic and Glycosylation Site Specific Data in GlyGen

Raja Mazumder

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Genomic variation data such as polymorphisms, cancer mutations and also data from mutagenesis experiments when mapped to glycosylation sites can reveal if such variations lead to gain or loss of glycosylation sites. Such information can provide clues to the functional impact of such variations, assist in biomarker discovery, and explore potential disease contexts. GlyGen provides an integrated view of such data at the protein level and also allows advanced search mechanisms to identify proteins with such loss or gain of glycosylation sites due to single nucleotide variations (SNVs).

The talk will provide an overview of the GlyGen data model, data integration workflow, and frontend tools (Super Search) that enables users to search for such interesting sites and compare them with homologs (using BLAST, CLUSTAL) and data from cross-referenced resources to gain detailed knowledge of a protein.

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Applying Transcriptomics to Study Glycosylation at the Cell Type Level

Hiren J. Joshi

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Copenhagen Center for Glycomics
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Copenhagen, Denmark

The complex multi-step process of glycosylation occurs in a single cell, yet current analytics generally cannot measure the output (the glycome) of a single cell. The glycome is not directly translatable from the genome as it is synthesised through the activity of a metabolic network of enzymes. Previously, we assembled information on the contributions of glycosylation and modification enzymes to the glycome into an atlas of cellular glycosylation pathways [1,2], which we can use to infer glycosylation capacity from the repertoire of expressed enzymes.

Here, we tested usage of single cell transcriptomics to break the single cell barrier for glycomics by estimating glycosylation capacities in individual cell types [3].

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Studying the Mechanism of Bacterial Protein Glycosylation

Marthe T. C. Walvoort

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Stratingh Institute for Chemistry
Groningen, The Netherlands

Protein glycosylation is an abundant post-translational modification. While it has long been assumed to be specific to eukaryotes, it is now well established that also bacteria produce glycoproteins. Interestingly, in bacterial glycoproteins, there is a lot more structural variation of both the glycan and the linkage to the protein. The glycans of bacterial glycoproteins have been associated with virulence, and therefore may present a novel target for antibiotic strategies.

In our research, we aim to understand how bacteria synthesize these glycoproteins, and I selected two types of glycoproteins for this lecture. The first type are 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, which is a highly unusual mechanism for enzymatic glycosyl transfer.

The second type of glycoproteins are EF-P proteins produced by *Neisseria* and *Pseudomonas* bacteria, amongst others, which are important for ribosome function. EF-P proteins are found to carry an unusual arginine-linked rhamnose moiety. Using a collection of synthetic peptides, we revealed that the glycosyltransferase, responsible for transferring the rhamnose unit, specifically recognizes a beta-hairpin fold. This structural requirement may inspire the future development of novel inhibitors.

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Elucidating Bacteria Toxin and Mucin Binding Domains – Specificities Towards Mucin-O-Glycopeptides

Sandra Behren[1], Jin Yu[2], Christian Pett[1], Manuel Schorlemer[1], Ulrika Westerlind[1]

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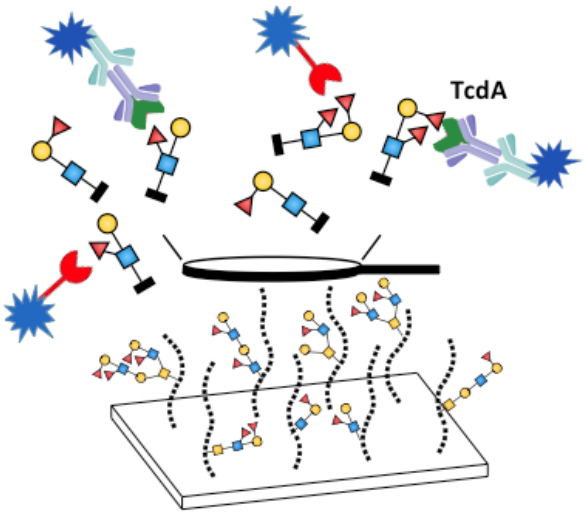
Mucins are densely O-glycosylated membrane-bound or secreted proteins ubiquitously found on the epithelial cell surface [1]. They are part of the innate immune system and play major roles as protective barriers to defend the host against invading pathogens [2]. However, bacteria and viruses have co-evolved with the human host and developed strategies to promote virulence for instance by adhering to carbohydrate ligands on the host cell-surface via pathogenic lectins. In order to improve our understanding of the pathogenic adhesion processes on a molecular level, we study the interactions between bacteria adhesion proteins and O-glycans presented on mucin tandem repeat peptide backbones.



Through the use of carbohydrate binding bacteria toxins such as Toxin A, interactions with glycans at the epithelial cell-surface, results in internalization of enzymes that promote virulence by modulation of the intracellular host GTPases.

To enable penetration of the mucus layer and make the epithelial cell-surface accessible, bacteria further secrete glycosidases and mucinases.

In recent years we have through a chemoenzymatic approach prepared extensive libraries of mucin tandem repeat glycopeptides consisting of different O-glycan core structures modified with LacNAc, LacdiNAc, sialylation and fucosylation [3-5].

In Limburg we will present our latest results elucidating binding interactions between mucin O-glycopeptides and the mucinase glycan binding domains as well as binding studies of toxin A originating from different pathogenic bacteria strains.

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Poster Abstracts

Tuesday

Poster #1

Exploring Protein *N*-glycosylation Alterations between Wild-type and Mutant Forms of Irisin

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Introduction: Irisin, an exercise-induced hormone from membrane protein FNDC5 secreted by muscle, is heavily glycosylated with 100% conserved sequences between mouse and human. Irisin conveys multiple physiological benefits, including thermogenesis of white fat tissue, bone remodeling, improved cognition, and resistance to neurodegeneration. Irisin mediates its effects via αV integrins targeting a unique binding site distinct from the canonical integrin ligands. Mass spectrometry analysis identified three *N*-linked glycosylation sites. Interestingly, while two (N8/N53) of the *N*-glycosylation sites are 100% glycosylated, the third non-canonical site (N45) is barely (<2%) glycosylated in endogenous irisin but 100% glycosylated in recombinant irisin produced from mammalian cells. The aim of this work was to study differences between wild-type and mutant forms of irisin using mass spectrometry.

Method: Irisin portion (32aa-132aa) from full-length gene FNDC5 cDNA was cloned into pcDNA3.1 vector downstream of the mouse IgG signaling peptide to force irisin secretion expressed in HEK293T cells. An endogenous-mimetic mutant of irisin was obtained by mutating the N45 glycosylation site. Irisin was C-terminally FLAG-tagged for affinity purification from cell culture medium and subsequently purified via buffer exchange/gel filtration steps. Purified protein samples (~100 µg) were digested with trypsin, *N*-glyco(peptides) were enriched using HILIC columns (20 µm, 300 Å) and analyzed on a 90-min gradient in triplicate using a C18 reversed phase column (100Å, 3 µm, 75 µm X 150 mm) on an Exploris-480 FAIMS system in positive mode. Collision-induced dissociation with data-dependent acquisition strategy was used and data analyses were done using Peaks and GlycReSoft.

Result: Prior to mass spectrometry analysis, affinity analyses were performed to compare wild-type and mutant irisin profiles. The N45Q mutant showed ~10-fold improved affinity for integrin receptor biochemically, and >5-fold increase of bioactivity when testing integrin signaling induction in cultured HEK293T ectopically expressing αVβ5 integrin and suppression of melanoma cells. To support these findings, we used mass spectrometry as an orthogonal approach to explain structurally and molecularly how glycosylation affects the bioactivity of irisin. The FAIMS device separates packets of ions based on their mobility along with *m/z*, which allowed us to perform a deeper coverage for *N*-glycopeptides. This approach complemented the affinity analyses well, by showing an increased *N*-glycosylation level in the mutated irisin (>150 glycopeptides) compared to wild-type irisin (<100 glycopeptides). While the major glycoforms occupied by irisin were found to be complex-type *N*-glycosylation, irisin was found to be decorated with paucimannose, oligomannose and hybrid-type glycans. A distinct site-specific micro-heterogeneity profile was observed between wild-type and mutant irisin; different abundances of tri-, tetra- and penta-antennary type complex glycoforms were confirmed on the mutant irisin with respect to the wild-type irisin. Additionally, the use of different compensation voltages in the FAIMS experiments allowed us to identify and quantify several low-abundance *N*-glycopeptides on the mutant irisin. With this in-depth *N*-glycoproteome profiling of irisin, we mapped the individual glycoforms for each site to explore the molecular dynamics and functional roles of *N*-glycosylated irisin.

Novelty: This is the first study to generate a comprehensive *N*-glycoproteome profile and compare between wild-type and mutant irisin.

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Poster #2

Towards the Development of a Spatio-temporal O-Glycosylation Reporter System

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Mucin-type O-glycosylation is amongst the most complex glycosylation modes, initiated by the addition of *N*-acetylgalactosamine (GalNAc) to Ser/Thr residues on the protein backbone. A family of 20 Golgi-resident GalNAc-transferase (GalNAc-T) isoenzymes catalyse the transfer of GalNAc onto glycoproteins. GalNAc-Ts demonstrate substrate specificities alongside a high degree of redundancy, making elucidation of their individual roles difficult. The biosynthesis of specific glycoforms at particular locations on the protein backbone is crucial for O-glycoprotein function. Aberrant mucin-type O-glycosylation is implicated in myriad pathologies, including cancer. A complex interplay between GalNAc-Ts appears to modulate O-glycoprotein maturation, with different GalNAc-Ts acting in concert to direct sequential O-GalNAc glycosylation at different residues on the protein backbone. A more detailed picture of the intermediate species in this process will shed light on the nature of O-glycoprotein maturation.

Herein, we outline a platform to probe intermediates in mucin-type O-glycoprotein maturation. We employ state-of-the-art bump-and-hole engineering of GalNAc-Ts, in conjunction with next generation chemical tools to trace individual GalNAc-T substrate preferences within a native biological context. In combination, O-glycoprotein intermediate species trafficking through the Golgi will be isolated and characterised. Thus, a temporal view of O-glycoprotein maturation will be elucidated. It is hoped that the findings of this research will inform our understanding of O-glycoprotein maturation in the secretory pathway, alongside the mechanisms underpinning GalNAc-T interplay. These findings may yield new diagnostic tools and open doors to new therapies for aberrant glycan-associated pathologies.

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Poster #3

Modelling the Processing of Glycans on Secreted Glycoproteins

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N-glycosylation is important for the pharmacological properties and efficacy of many therapeutic proteins. However, glycosylation is a complex non-templated process which results in a highly heterogeneous population of glycan structures, preventing precise control over the resulting glycan distribution. Therefore, a method to rationally engineer glycosylation with increased homogeneity and advantageous glycan structures would be highly beneficial to the pharmaceutical industry.

We have experimentally validated a computational model of glycan biosynthesis. This combines stochastic simulation with Bayesian fitting, to reveal the organisation of the glycosylation machinery when simulating biosynthesis of the cell's *N*-glycan repertoire. This computational modelling tool was recently adapted to investigate the relationship between organisation of the glycosylation machinery in the Golgi and the glycoform distribution of therapeutic IgGs.

We present use of the modelling to investigate differences in biosynthetic determinants influencing the overall cellular glycan profile (CGP) vs. glycosylation of a single glycoprotein produced by the same cell. We opted to use the therapeutic IgG Herceptin as a model, as the glycan on IgGs is known to undergo more restricted processing in the Golgi than many other glycoproteins. Both CGP and Herceptin glycan profiles were acquired in WT CHO cell lines, as well as in mutants with altered glycosylation. Through modelling the CGP glycosylation changes between WT and mutants, we identified relative shifts in enzyme levels and localisations. We then assessed how these shifts in enzyme organisation can predict changes in Herceptin glycosylation.

Applying the changes between WT and mutant cells did accurately predict changes to the abundance of large highly-processed glycans, indicating that galactosylation at the cellular levels is translated well onto galactosylation of Herceptin. However, intermediate glycan abundances are not predicted well using this method, implying differences in the efficiency of medial Golgi processing steps for Herceptin compared to an “average” glycoprotein.

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Poster #4

Mapping Glycosyltransferases Glycosylation Sites and Substrate Preferences *via* Glycoproteomics

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The characterisation of the glycoproteome is particularly challenging due to the interplay of glycosyltransferases (GTs) and the presence of both macroheterogeneity (site occupancy) and microheterogeneity (several possible glycoforms at a specific site). The study of such complex biological system is made possible by specific chemical and biological tools [1]. Among these, we employ the bump-and-hole (BH) engineering strategy [2] to generate mutant GTs that contain an enlarged active site. This modification of the catalytic pocket is obtained by mutating large 'gatekeeper' amino acids into smaller ones. This allows the modified enzyme to accommodate a bulkier biorthogonal monosaccharide that gets incorporated when cells are fed leading to highly selective functionalisation of glycoproteins in living cells. Mass spectrometry, and specifically bottom-up glycoproteomics, is a powerful tool for the study of the glycoproteome as it allows for the characterisation of intact glycopeptides and can provide information about site-specific localisation.

This work is primarily focused on the optimisation of methods for glycoproteomics [3,4]. Main efforts have been put on crucial steps of the glycoproteomics workflow such as enrichment, clean-up, fragmentation procedures and data analysis. By developing and optimising glycoproteomics techniques, we aim to map glycosylation sites for glycosyltransferases of interest to better understand their function. Knowing GTs substrate preferences and glycosylation sites will help us shed light into their biological role.

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Poster #5

Evolution of the Interactions between Rotavirus VP8* and Histo-blood Group Antigens: Insights from Computational Studies

Roberto Cárcamo-Calvo[1,2], Nararet Pena-Gil[1,2], Noemi Navaroo Lleó[1], Cristina Santiso-Bellón[1,2], Roberto Gozalbo-Rovira[1,2], Jesús Rodriguez-Dìaz[1,2]

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Rotavirus is the leading cause of severe acute childhood gastroenteritis, responsible for more than 128,500 deaths per year, mainly in low-income countries. However, since the introduction of rotavirus vaccines, the diversity among the most common genotypes has increased considerably. Even so, it is estimated that the P[8] genotype is responsible for more than 80% of human infections Within this genotype, four different lineages have been described phylogenetically: P[8]- lineage I, P[8]-lineage II, P[8]-lineage III and P[8]-lineage IV. On the other hand, rotavirus resistance to infection apperars to be genotype-dependent, and it is mainly mediated by the interaction between histo-blood group antigens and rotavirus distal spike VP4 portion. Nevertheless, despite some experimental results, molecular basis of host cell recognition by rotavirus are unclear. This study seeks to give insights into the consequences of such diversity between intra-genotype lineages on the recognition of target cells by rotaviruses and its potential consequences on novel vaccine development. The bioinformatic approach presented here has shown to properly reproduce the known interactions between the VP8* from rotavirus with its glycans receptors. We have been able to replicate binding preferences for secretory versus non-secretory and Lewis-positive antigens of the VP8* from the P[8] genotype. Moreover, molecular dynamics analysis revealed transient bindings between VP8* P[8] and the H1 antigen. Nonetheless, some weaknesses of the method were detected since molecular docking analyses were not able to predict the differences in VP8* binding between H1 antigen and its precursor as previously demonstrated experimentally.

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Poster #6

Fine-tuning the Spike: Role of the Glycan Shiel in the Structure/Dynamics of SARS-Cov-2S

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The SARS-CoV-2 spike (S) is a type I fusion glycoprotein, responsible for initiating the infection leading to COVID19. As a feature unique of SARS-CoV-2, the thick glycan shield covering the S protein is not only essential for hiding the virus from immune detection, but may have a functional role. These newly discovered functions of the glycan shield suggest the evolution of its sites of glycosylation is potentially intertwined with the evolution of the overall protein sequence to affect optimal activity. In this work we use multi-microsecond molecular dynamics simulations with different N-glycans at key functional sites, namely N234, N165 and N343. We also assessed the effect of a change in the SARS-CoV-2 S glycan shield's topology at N370, due to the recently acquired T372A mutation. Our results indicate that the structures of the N-glycans at N234, N165 and N343 affect the stability of the active (or open) S conformation, and thus its exposure and accessibility.

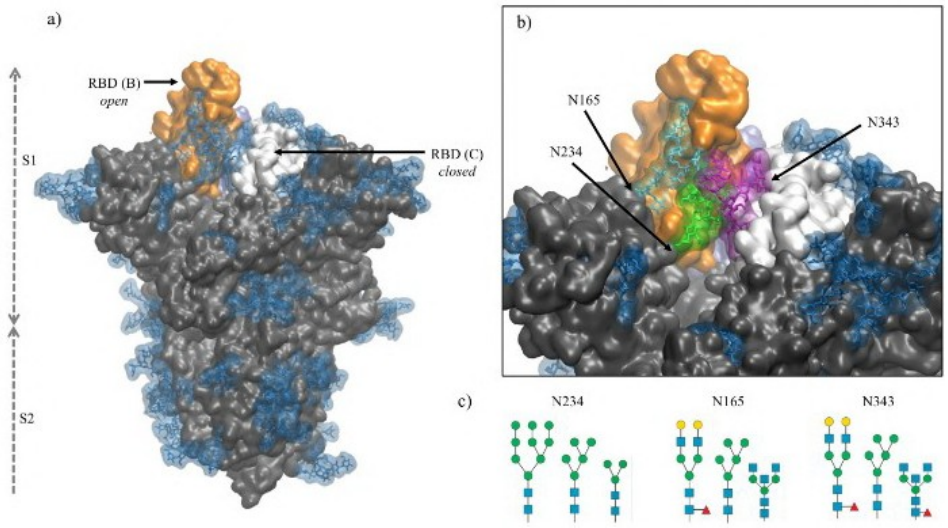

Figure 1. Illustrates the S 1/2 units of the spike protein in the open conformation and panel c) shows the SNFG representation of the variations in the glycan shield explored in the paper [see reference].

Furthermore, while glycosylation at N370 stabilizes the open S conformation, we find that the N370 glycan binds the closed receptor binding domain (RBD) surface, essentially tying the closed protomers together. These results suggest that the loss of the N370 glycosylation site in SARS-CoV-2 may have increased the availability of the open S form, perhaps contributing to its higher infectivity relative to CoV1 and other variants carrying the sequon. Finally, we discuss these specific changes to the topology of the SARS-CoV-2 S glycan shield through ancestral sequence reconstruction of select SARS strains and discuss how they may have evolved to affect S activity.

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Poster #7

Simple, yet Logical Terms for *N*-glycans to Communicate Isomer-specific Analysis

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With probably most, if not all, mammalian glyco enzymes now discovered, the enormous extent of the *N*-glycome can now be gauged. The art of separation and exact assignment of structures lags behind. For structures of modest size, isomer-sensible *N*-glycan analysis is nevertheless taking shape with the application of shape-selective chromatography and mass spectrometry. Porous-graphitic carbon chromatography with normalized retention times promises the highest level of structural selectivity. Nearly all of forty isomers of the composition Hex5HexNAc4Fuc1 adopted unique positions on the retention time vector [1, 2].

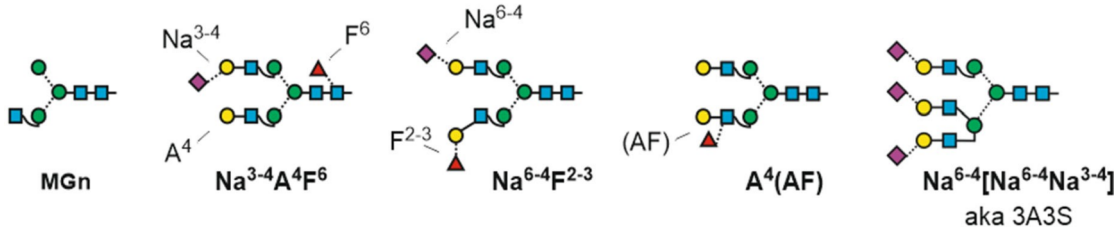
Work with this multitude of isomeric *N*-glycans made us realize the lack of codes that can be written and spoken and yet be unambiguous and understandable without external references. To this end we have elaborate the **pro**tein **gly**cosylation **an**alysis code, which lists the terminal residues of an *N*-glycan only relying on the certainty that the following structure is self-evident. Remaining ambiguities are eliminated by superscript numbers. Thereby, arm and linkage isomers, antennal architecture, fucose locations and more are unambiguously encoded with terms that can be scribbled on reaction vials.

The following basic rules apply:

- Terminal residues are read counter clockwise starting top left,
- Monosaccharides are depicted by one capital letter, modifications by a subsequent small letter

G … glucose	Gn … *N*-acetylglucosamine	F … fucose
A … galactose	An … *N*-acetylglucosamine	X … xylose
M … mannose	Na … N-acetylneuraminic acid	Ng … *N*-glycolylneuraminic acid

- Substituents to the β-galactose are linked to this residue by a hypen. The terms -A4 or -A^3 are abbreviated to the superscripts $^{-4}$ or $^{-3}$. Thus: Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-2)Man(a1-6).... is written as Na^{6-4}.
- Bisected GlcNAc is indicated by "bi" and is always listed as the last extension term.
- Termini of branched antennae are set in round brackets.
- Square brackets group the antennae of tri- or tetra-antennary glycans.



The **proglycan** system appears appropriate wherever fully defined structures have to be clearly, yet briefly described. It does not aim at fully displacing existing naming systems, which have their merits for larger, insufficiently defined *N*-glycans.

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Poster #8

Towards Clinical O-glycomics: Application for Osteoarthrithis

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Clinical glycobiomarkers to improve diagnostics of diseases and prognostics after treatment is an unexplored territory. Despite that glycosylation is often reported to be pathologically altered, the transfer from discovery using high end non-targeted -omics mass spectrometric (MS) based technology into the clinics is often difficult. In the clinics, robust technologies such as ELISA and target mass spectrometry are readily utilized. Our group is committed to approach Osteoarthritis (OA) from an O-linked glycobiological angel[1, 2]. OA is the most prevalent human disease in the world and is also one of the most disabling, characterized by the breakdown of cartilage in the joints. Defect lubrication due to altered O-linked glycosylation is part of the OA pathology that contributes to the disease-associated degradation of the joint. The O-glycome and O-glycoproteome of synovial fluid from osteoarthritic patient was revealed using MS. Pathological changes, including shortening of the O-linked glycans was identified. In order to develop a more clinical friendly screening we adopted Selected Reaction Monitoring (SRM) MS for the O-linked glycans for detecting changes in the glycosylation of OA patients' synovial fluid. Finally, we developed a sensitive Lanthanide Fluorescent Immunoassay (DELFIA) based on lectin nanoparticle technology to be able to detect glyco-altered synovial O-glycoproteins that leaked out from the joint to the plasma.

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Poster #9

Exploring the Glycoproteome of the Sheep Myiasis Fly, *Lucilia cuprina*, for Novel Vaccine Targets

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Ectoparasites, typically arthropods, inhabit the skin or skin surface of a host organism and can be detrimental to the host directly and/or and can act as disease vectors. Flystrike (cutaneous myiasis) on sheep is a major sheep health and welfare problem in Australia caused by *Lucilia cuprina* (Australia Sheep Blowfly). The fly lays eggs on live sheep, which hatch soon after and the larvae feed on the sheep causing physical damage and predisposing the area to secondary bacterial infections. In Australia, the economic consequence of flystrike in sheep is due to losses of production (liveweight gain and wool) and costs of prevention and control, and is more than AU$320 million annually. With the continued development of resistance to insecticides, a key approach to controlling insect parasites is through developing effective vaccines that modulate the host immune system to stop or impede the growth of the parasite. Secreted proteins that are integral components of the peritrophic matrix (PM), a lining of the midgut composed of chitin and proteins are candidate antigen targets for vaccine development and have shown efficacy in flystrike vaccine research.

Peritrophins, a key class of proteins that constitute the PM are associated with the structural and functional integrity of the PM structure. Previously, vaccination with a core PM peritrophin with a mucin-like domain isolated directly from blowfly larvae was shown to result in 60% inhibition of larval growth in *in vitro* larval feeding assays whereas the recombinant form of the antigen produced in bacteria or lepidopteran insect cells resulted in less than 20% inhibition [1].

The reason for the difference in vaccine efficacy of the antigens may be associated with protein folding and post- translational modifications, such as glycosylation, as the recombinant systems differ from production of proteins in vivo in *L. cuprina*. Here, we describe the first reported analysis of the complex glyco/proteome of the *L. cuprina* PM and compare the native glycosylation patterns to recombinant PM antigens.

We also use transcriptomics to predict the glycosylation capacity of the cardia, the specialist PM producing organ at the start of the anterior midgut compared to other blowfly organs to better understand the glycosylation patterns of the PM. This understanding of the glycosylation signatures of the PM is being used to help inform current vaccine development efforts.

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Poster #10

Elucidating the *O*-glycan Structure of the Novel Periodontal Health-associated Bacterium *Tanerella serpentiformis*

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Tannerella forsythia, was recently discovered to play a remarkably health-associated role in the oral biofilm community. For instance, *T. serpentiformis* shows a decreased invasiveness of host cells compared to *T. forsythia.* [1]. Furthermore, the striking difference in biofilm behavior between the two *Tannerella* species is accompanied by a notable lack of nonulosonic acid biosynthesis genes in the *T. serpentiformis* glycosylation gene cluster. Nonulosonic acids are well-known for their pivotal role in bacterial pathogenicity and it has been shown that a modified nonulosonic acid is characteristic of the S-layer O-glycan of pathogenic *T. forsythia* strains, where the S-layer is a virulence factor [2, 3]. We hypothesize that differences in the glycans displayed on the *T. serpentiformis* cell surface contribute to the bacterium's dissimilar pathogenicity potential and lifestyle.

In this study, we elucidated the structure of the *T. serpentiformis* S-layer O-glycan by NMR and MS analysis and found that it is present in two main glycoforms, which differ only slightly in linkages and composition. We identified seven different monosaccharides that make up a branched deka- or undekasaccharide and carry (non-)carbohydrate modifications. There are four to five fucoses present in each glycoforms, which are O-methylated and in one case modified with an NH_2 group, which has been described only once in the literature, so far. Importantly, no nonulosonic acid is present in the O-glycan and a hexose serves as linking sugar to the protein.

Moreover, we could identify over 200 individual heavily glycosylated proteins in *T. serpentiformis*, including the bacterium's abundant surface layer glycoproteins. Beyond that, our data support the phylum-wide postulated O-glycosylation amino acid motif [4] based on bioinformatics analysis of MS data against a background of non- glycosylated sequences.

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Poster #11

Wholistic Heparan Sulphate Oligosaccharide Profiling Using Top-down GAGomics

Marissa L. Maciej-Hulme, Jandi Kim, Elija Roberts, Yiqing Zhang, I. Jon Amster, Johan van der Vlag

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Heparan sulfates (HS) are a group of heterogenous linear, sulfated polysaccharides that play a role in many diseases including cancer, cardiovascular, and kidney diseases. However, the structural variety of HS has greatly challenged the development and utility of HS analytics for native structures, leaving a significant gap in HS technologies for clinical application. Mass spectrometry-based profiling with bioinformatics offers a top-down approach that can retain variety in large data sets. Using healthy human plasmas, we developed a glycoprofiling approach for native HS oligosaccharides, which retains the structural complexity of each individual HS chain and generates an HS 'index' (or Heparan-ome) for each patient. Analysis of kidney disease patient plasmas revealed a new subset cluster (20%, 4/20) of membranous glomerulopathy (MG) patients with distinct HS profiles, highlighting the potential of HS glycoprofiling as a powerful new approach for HS biology, and future development into non-invasive clinical diagnostics for MG and other HS-mediated diseases.

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Poster #12

Development of a Machine Learning Framework to Extract the Biomarker Potential of IgG *N*-glycans

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Aberrant protein glycosylation is involved in disease pathophysiology. Identification of disease- specific glycan alterations in plasma proteins can provide valuable information on disease diagnosis and monitoring, as well as patient stratification. The ever-growing field of glycomics has enabled the unprecedented generation of glycosylation data for different disease types, which can complement clinical data to aid the discovery of biomarkers. This increased data availability gives rise to the need for developing robust data analysis and machine learning tools to enable the discernment of glycan-based biomarkers and their relationship with disease outcomes. This work proposes a comprehensive interpretable machine learning framework developed using colorectal cancer (CRC) data (Vuckovic *et al.*, 2016).

In particular, the dataset comprised the relative abundances of 24 IgG *N*-glycans and known covariates such as age and gender for 1,413 CRC patients and 538 controls. Limitations of the dataset include an imbalance in the number of controls versus the number of patients over the age of 60 years. To overcome the class imbalance with respect to healthy controls, a modified version of the Synthetic Minority Over-Sampling Technique (SMOTE) is employed, which incorporated the knowledge for the age and gender distributions across healthy and patient samples. Regarding predictive modeling, five machine learning methods are trained on preprocessed data, namely Random Forest (RF), Support Vector Machines (SVMs), Logistic Regression (LR), Extreme Gradient Boosting (XGBoost) and Soft Voting Ensembles (SVEs). To reliably assess generalization performance, nested cross- validation (NCV) is employed for hyperparameter tuning and model evaluation.

Finally, biomarker potential is evaluated using permutation feature importance, a model-agnostic method for interpretability that enables the identification of the most influential *N*-glycan features in the classification of disease outcomes.

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Poster #13

Serum *N*-glycan Biomarkers Predict Patient Response to Vedolizumab Treatment for Crohn's Disease

Georgia Elgood Hunt[1], A. Adams[2], T. Senard[1], R. Gardner[1], W. de Jonge[3], A. Li Yim[3], A. Noble[2], J. Satsangi[2], V. Joustra[3], G. D'Haens[3], D. Spencer[1]

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Introduction: A monoclonal antibody, Vedolizumab (VDZ), is used to treat Crohn's disease (CD), which has proven to be efficacious in phase 3 clinical trials[1] and in real-world studies [2]. Glycosylation changes have been associated with CD, but have never been studied as potential predictors of remission to biological therapy [3]. A comparison of serum *N*-glycan profiles between responders and non-responders to VDZ was used to detect potential biomarkers to response.

Method: Serum samples before (t1) and after (t2) initiation of VDZ were taken from 58 patients with CD. Response to VDZ was assessed by a combination of clinical and endoscopic outcomes and was observed in 34 patients. Glycans were detected by hydrophilic interaction liquid chromatography coupled with mass spectrometry. 89 direct traits met the criteria for quantification via LaCyTools [4]. 21 derived traits were calculated by combining direct traits with structural similarities. Correcting for age and sex, traits were tested across responses using logistic regression to determine which traits at t1 are predictive of response (p-value < 0.05) and traits at t2 are markers of response (p-value < 0.05). Five-fold cross-validation tested on a variety of classifiers was used to derive the optimal predictor of response.

Results: Glycans, MAN6 (0.65 AUC) and FA2G2S2 (0.7 AUC) were higher in responders prior to treatment and moderate predictors of response. Congruent to previous studies, galactosylation levels increased in responders after treatment.

A clinical marker (F-calprotectin) proved to be a weaker predictor than direct traits (0.43 AUC). Overall, 17 direct and 4 derived traits were markers of response and collectively proved to be good predictors of response (0.80 AUC).

Conclusions: This study has demonstrated multiple *N*-glycans are potential predictors of VDZ response, with the possibility to guide clinicians in the most appropriate form of treatment. These results require validation by larger cohorts.

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Tuesday

Poster #14

Integrated Glycomics and Genomics Reveals a Role for Plasma Protein and IgG *N*-glycosylation in Type 1 Diabetes Development

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Individual variation in *N*-glycosylation and its role in T1D onset is mainly unknown. In this study the aim was to explore *N*-glycome and genome interplay at the onset of T1D. Plasma protein and IgG *N*-glycans were chromatographically analysed in a study population comprising 1917 children and adolescents (0.6-19.1 years) with new-onset T1D from the DanDiabKids registry. In the follow-up study results for 188 of these participants were compared with those for their 244 unaffected siblings. A total of 1105 new-onset T1D patients were genotyped at 183,546 genetic markers, testing these for genetic association with 24 IgG and 39 plasma protein *N*-glycans. Significant genetic associations were validated in 455 samples.

Our study showed that T1D onset was characterised by an increase in plasma and IgG high-mannose and bisecting GlcNAc structures, a decrease in monogalactosylation, and an increase in IgG disialylation. Models including age, gender and *N*-glycans yielded significant discriminative power between children with T1D and their unaffected siblings, with AUCs of 0.915 and 0.869 for inclusion of plasma and IgG *N*-glycans, respectively.

This study identified novel associations that were not previously reported for the general European population: 1) novel genetic associations of IgG glycans were identified for SNPs on chromosome 22 close to candidate gene *MGAT3*; these include core fucosylated digalactosylated disialylated IgG *N*-glycan with bisecting GlcNAc and its asialylated version; 2) novel genetic locus on chromosome 19 was associated with plasma protein *N*-glycosylation, the complement C3 gene (*C3*). Identified *C3* missense variants are often coinherited with another T1D risk-associated variant and the associated Man9 glycan resides on a domain involved in the C3 pathogen binding. This study identified distinct N-glycosylation at T1D onset, novel genetic variants contributing to these changes and potential underlying molecular mechanisms.

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Poster #15

Linking Glycosylation to Function

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Recent updates of GlyConnect include a Linked Data version of the database. This allows "federation" or centralized querying of a number of resources using common identifiers. In addition, information can be shared and automatically read by computers.

For example, through the use of UniProt accession numbers, which are used to annotate glycoproteins in GlyConnect, it is possible to access related annotations in UniProt and/or neXtProt, and link these to the associated glycan structures as well as molecular functions and reactions (Rhea and interact databases).

We present the update to our GlySTreeM ontology for glycan structures which now includes mapping to a human determinant list from Cummings *et al* (PMID 19756298). These determinants (also called glycan epitopes) have been shown to be important in many aspects of biology including signalling, recognition events and immune responses. The annotation of glycan topologies with determinants (also called epitopes) as part of the GlyConnect resource can better aid interpretation of glycome profiles.

The structure/epitope mapping is recorded both in GlyConnect relational database and the GlySTreeM triple store. In GlyConnect, the mapping is used to populate the Octopus Search Tool, and in the GlySTreeM SPARQL Endpoint, it is possible to construct queries to search for epitopes and/or the glycans that contain those epitopes.

As a use case, we used federated queries with the neXtProt database to pull out all pairs of interacting proteins where at least one of the partners contain a glycosylation site (as validated by GlyConnect). The glycans that are associated with the interaction site were compared using the annotated epitopes to investigate the types of glycosylation that are found.

Federated queries will also be possible with UniLectin, the database of lectins, as an RDF version is currently being constructed. Again, the glycan ligands (epitopes) of certain ligands are well characterised and these can be used to map against the set of glycans found in GlyConnect.

Users can either access the resources by API (GlyConnect), RDF endpoints (GlyConnect RDF & GlySTreeM) or by using our new query interface (coming soon).

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Poster #16

Bioinformatics Analyses of Nematode Hexosaminidases

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Hexosaminidases are ubiquitous enzymes with multiple roles in glycoconjugate metabolism as they remove non-reducing terminal β-*N*-acetylhexosamine residues from glycans, glycolipids, glycoproteins and glycosaminoglycans. Amongst many functions in non-invertebrates, hexosaminidases are involved in maturation of *N*-glycans.

Bioinformatic analysis focused on phylogenic reconstruction, which indicates that these hexosaminidases evolved within the nematode branch. All nematode hexosaminidases share a common ancestor, and within the class there has been gene duplication and specialization. Furthermore, an analysis of transcriptome data conducted by Hutter in 2009 revealed that *C. elegans* expresses five different hexosaminidases (HEX-1, -2, -3, -4, -5) during various stages of its embryonic and post-embryonic development.

Homology-based 3D-structure prediction of hexosaminidases from *C. elegans* and *T. suis* showed no significant differences in the substrate binding pocket. Recently, we acquired a crystal of HEX-3 *T. suis* and could get diffraction at a resolution of 2.5 Å. However, the structure does not solve the question of the enzymes' specificity.

In vitro enzyme tests of HEX-3 *T. suis* showed that the enzyme favors pNP-β-GalNAc, but still can remove GlcNAc from the *N*-glycan substrate GnGn-PA. Thus, this Trichenellid HEX-3 enzyme is functionally more similar to HEX-2 and HEX-3 from *C. elegans* than to HEX-4. The latter is a Golgi enzyme can remove terminal GalNAc even from structures in which the underlying GlcNAc was substituted with phosphorylcholine.

Overall, these nematode enzymes are not only interesting due to their roles in glycan biosynthesis, but are useful tools as a complement to mass-based glycomic workflows.

Tuesday

Poster #17

A Bacterial Mannose Binding Lectin as a Tool for the Enrichment of C- and O-mannosylated Peptides

Hermann J. Hütte[1], Birgit Tiemann[1], Aleksandra Shcherbakova[1], Valerian Grote[2], Marcus Hoffmann[2], Lorenzo Povolo[3], Mark Lommel[4], Sabine Strahl[4], Sergey Y. Vakhrushev[3], Erdmann Rapp[2,5], Falk F.R. Buettner[1], Adnan Halim[3], Anne Imberty[6], Hans Bakker[1]

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[6]Université Grenoble Alpes, CNRS, CERMAV, Grenoble, France

We can detect C-mannosylated peptides from purified proteins by mass spectrometry (MS). However, identification of these from complex biological samples is challenging. Lectin affinity chromatography is a common method to enrich specific glycopeptides, which increases the chance of detection by LC-MS/MS. For C-mannosylated peptides, there was no such tool available. We explored the used of the α-mannose-specific *Burkholderia cenocepacia* lectin A (BC2L-A) and demonstrated that, in addition to its previously shown binding to mannose terminating *N*-glycans, this lectin is able to retain C- and O-mannosylated peptides. We first used tryptic peptides derived from recombinantly expressed and purified proteins. BC2L-A binds C-mannosylated peptides independent of the number of C-mannoses and the presence of O-fucose on the same peptide. We also observed strong binding of O-mannosylated peptides to BC2L-A. In contrast to other mannose-binding lectins like Concanavalin A (ConA), BC2L-A recognizes a single mannose and peptides can be eluted using the calcium chelator EDTA.

Applying BC2L-A affinity chromatography, we were able to enrich C-mannosylated peptides from complex samples of tryptic digests of HEK293 and MCF10A whole cell extracts. This led to the identification of novel C-mannosylation sites on TSR-containing proteins but also on other protein families, including sites beyond the known C-mannosylation consensus sequence. In conclusion, BC2L-A enabled specific enrichment of C-mannosylated peptides, not shown by any other lectin. In addition, due to its advantageous properties, BC2LA potentially is superior to other lectins for binding of other α-mannose-terminating glycans as well.

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Software Abstracts

Tuesday

Software #1

Privateer – Validation of Modelled Glycosylation in the 3D Structural Biology Frontier

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Protein glycosylation is a common modification that involves the enzymatic transference of oligosaccharides onto protein. Glycosylated proteins are estimated to make up between 50% and 70% of the human proteome[1]. Accurate atomic structures, obtained experimentally via X-Ray Crystallography and Cryo-EM, are a prerequisite for mechanistic insights. Unfortunately, heterogeneity, mobility and complexity of glycans in glycoproteins have been, and currently remain, significant challenges in structural glycobiology [2,3]. Both glycan compositional and conformational anomalies in Protein Data Bank Entries depositions have been reported [4,5].

The Privateer software was developed as a tool to detect and address most problems affecting carbohydrate structures in the PDB. At the core of the software, is the validation and support for re-refinement of carbohydrates to ensure lowest energy conformations[6]. Recently, new features have been added to detect the modelling of improbable glycan compositions on target glycoproteins [7]. For every detected glycan in a structure, Privateer queries the GlyTouCan and GlyConnect databases [8-10], matching modelled glycans to glycoinformatics records via the WURCS notation. If a glycan is not detected on the databases, Privateer is able to return similar, but compliant structures, therefore highlighting potential issues within modelled glycan compositions [11,12]. Additionally, basic automated modelling capabilities have been included to allow for glycan grafting from donor PDB files onto receiver PDBs containing potential glycoproteins. This functionality has been used to demonstrate the amenability of AlphaFold models for glycosylation using a library of structurally equilibrated glycan blocks, obtained from molecular dynamics [13,14]. Thanks to the Python bindings to the C++ backend, new and old Privateer features can be readily integrated into other software pipelines, in terms of bridging the fields of structural biology and glycoinformatics.

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Tuesday

Software #2

Automated Glycopeptide Data Processing and Curation in High-sensitivity Applications

Tamas Pongracz, Sterre Siekman, Steinar Gijze, Manfred Wuhrer and David Falck

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Robust and automated glycopeptide quantitation from liquid chromatography - mass spectrometry (LC-MS) data is a challenge that is as yet insufficiently addressed by commercial software. In 2016, we therefore established LaCyTools [1], an open-source software package for automated processing of LC-MS glycopeptide data, which has been adapted and regularly used by several labs around the world. The software performs automated retention time alignment, spectral calibration, analyte quantitation and calculation of quality metrics. LaCyTools is characterized by a unique focus on speed and robustness, and is particularly suitable for the analysis of samples with highly similar sets of analytes, varying in relative abundance. Boosted by its low-hurdle graphical user interface, LaCyTools is a highly efficient tool for processing of large datasets (hundreds to thousands of samples), targeting glycosylation of single proteins and protein families with throughput-optimized methods of limited chromatographic resolution. LaCyTools has been maintained, updated and integrated, for example with workflows including analyte identification on the MS and MS/MS levels [2,3]. However, several important steps at the end of the workflow were still manual.

Ever larger sample numbers or ever more challenging applications, such as the glycoprofiling of antigen-specific antibodies [4], demanded further automation. We addressed this bottleneck by developing GlycoDash, a Shiny-based web application. The software incorporates several options for spectral and analyte curation, re-normalization, metadata linking, and visualization and reporting capabilities. Both tools greatly support adherence to FAIR principles, as, for example, output is provided in accessible standard formats and contains essential metadata. In the future, we aim to add advanced visualization and statistical analysis features.

The integrated processing using LaCyTools and GlycoDash will be demonstrated on challenging applications, such as the profiling of antigen-specific antibodies using our GlYcoLISA protocol.

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Tuesday

Software #3

A Demonstration of CarbArrayART – a Tool for Glycan Microarry Data Management and Reporting

Yukie Akune-Taylor[1], Sena Arpinar[2], Yan Liu[1], René Ranzinger[2], and Ten Feizi[1]

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Glycan microarrays are essential tools in glycobiology and are being widely used for assignment of ligands in diverse glycan recognition systems. We have developed a software tool, called Carbohydrate microArray Analysis and Reporting Tool (CarbArrayART) [1], to address the need for a distributable application for glycan for day-to-day use in glycan microarray data management. CarbArrayART is designed for recording, storing, processing, presenting and reporting of any slide-based glycan microarray experiment.

The focus of this demonstration on CarbArrayART will be:

(i) Entry tools for glycan microarray-related data which are compliant with the guidelines of MIRAGE (Minimum Information Required for A Glycomics Experiment) [2]

These include:

- Glycan probes
- Subarray layout
- Slide layout
- Sample metadata
- Experiment metadata
- Quantified array data (GenePix Result or Proscan files)
- Other data to archive such as the raw images from the scanner, related publications and associated experiment results (MS, Gel images etc).

(ii) Display tools for processed data

Presentation of microarray data as:

- Histogram charts and tables with filtering and sorting functions according to pre-defined monosaccharides, motifs and backbone types
- Tables containing multiple data sets for comparison
- Heatmaps for single and multiple experiments using the Excel file included in the CarbArrayART software package.

(iii) Tools for data export and import

- Data export using Word, PDF and Excel formats including all the metadata related to the experiment.
- Data export and import between other CarbArrayART users using GR file format (*.gr*) including raw and processed data files with all related metadata.

Software with online user's manual is accessible from http://carbarrayart.org.

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Tuesday
Software
#4

What's New on Glyco@Expasy?

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Glyco@Expasy is a portal destined to support new/experienced glycobioinformatics users in understanding the variety of available on-line resources. It was recently updated and so were the SIB databases and tools hosted on the Expasy webserver (celebrating 30 years of existence in 2023). Because glycoscience is fragmented, the SIB range is an attempt to cater for the distinct needs arising from each fragmented area.

In particular, GlyConnect contains structural knowledge relative to glycosylation (glycoconjugates) whereas UniLectin (glycan-binding proteins) provides the complement information supporting the study of glycan functional roles. In practise, GlyConnect users often do not intersect with the UniLectin ones and conversely. Yet, the very purpose of the Glyco@Expasy range is to re-establish such a lost connection between molecules that are interacting partners in real life, through the unification of resources.

The demo will show how glyco-data can be explored, linked and visualised with a few use cases.

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Tuesday
Software
#5

GlycoDomain Viewer, Glycopacity and Glyco.me

Hiren J. Joshi

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The GlycoDomainViewer is a comprehensive database of N- and O- glycosylation sites that has now been incorportated into the suite of tools available at https://glyco.me. This demo will give a taste of the glycoproteomic and glycogenomic tools available here.

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Tuesday

Software #6

GlyFinder: An Online Tool for Finding Glycans and Glycoproteins in the PDB

D.W. Montgomery, Robert J. Woods

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Tens of thousands of 3D structures of oligosaccharides have been deposited into the Protein Databank (PDB), representing hundreds of thousands of hours of effort by crystallographers. Yet, despite the critical importance of these structures in furthering the development of glycomimetic drugs, in explaining the activity of glycan-processing enzymes, and in providing a deeper understanding of the properties of glycoproteins and vaccines, they remain unnecessarily difficult to locate within the PDB. Part of this is due to limitations in searching for oligosaccharides on the PDB website, even after a recent carbohydrate remediation project completed by the PDB. While several databases have been reported that contain carbohydrate structural information extracted from the PDB, few offer flexible search capabilities, and even fewer provide independent assessment of data quality.

Here we present the GlyFinder and GlyProbity webtools (glycam.org/gf) and illustrate their application in locating oligosaccharides, carbohydrate derivatives, and glycoproteins stored in the PDB. We also highlight the utility of curating the data on the basis of the theoretical conformational (CHI) energies [1] of the glycosidic linkages.

We use these tools to examine structures of the conserved *N*-glycan core ($GlcNAc_2Man_3$) and its fragments. Because of the mechanism of *N*-glycosylation in eukaryotes [2], the linkages and monosaccharides in this core are the same across all classes of *N*-glycans (complex, high-mannose, hybrid). Despite this, we have found over 3,000 structures with differences in their core structure. These and other egregious structural errors certainly indicate an urgent need for further remediation of carbohydrates in the PDB to avoid the propagation of such fundamental errors.

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Tuesday

Software #7

GlyGen portal, glycan array data repository and the Glycoinformatics Consortium

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Please see Renés abstract on his talk on “The Glycan Array Data Repository” given on Thursday, 11:50.

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