POSTER PROGRAM

SESSION II: Glycan Regulation of Immunity and Infection I

Modulation of the innate immune responses to pathogenic Escherichia coli by paired Siglec receptors in humans and in mice

Poster #: LB1 || Abstract #: 229

Flavio Schwarz[1,2,3], Corinna S. Landig[1,2,3], Shoib Siddiqui[1,2,3], Ismael Secundino[1,4,7], Andrea Garcia-Bingman[1], Joshua Olson[4], Nissi Varki[1,5], Victor Nizet[1,4,6], Ajit Varki[1,2,3]
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Overlapping Substrate Specificity of Nucleotide Sugar Transporters in the Fungal Pathogen, Aspergillus Fumigatus

Poster #: LB2 || Abstract #: 230

Juliana Yeung[1], Mark Warwas[1], Brandon Kwok[1], Helen Croft[1], Lindsay Woof[1], Amrit Bath[1], Tysha Donnelly[1], Linda Pinto[1], Joe Tiralongo[2], Thomas Haselhorst[2], Margo M. Moore[1]
[1]Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada ; [2]Institute for Glycomics, Griffith University, Queensland, Australia

C elegans strains deficient in O-glycosylation show altered tolerances to a range of bacterial pathogens: Glycosylation impact on innate immunity

Poster #: LB3 || Abstract #: 231

Salil K. Ghosh[1], Michelle R. Bond[2], John A. Hanover[2], John F. Cipollo[1]
[1]Center for Biologics Evaluation and Research U.S. Food and Drug Administration; [2]National Institute of Diabetes and Digestive and Kidney Diseases

Vaccination with recombinant microneme lectins confers protection against experimental toxoplasmosis in mice

Poster #: LB4 || Abstract #: 232

Camila F. Pinzan[1], Aline Sardinha-Silva[1], Fausto Almeida[1], Livia Lai[2], Carla D. Lopes[1], Elaine V. Lourenço[1], Ademilson Panunto-Castelo[1], Stephen Matthews[2], Maria Cristina Roque-Barreira[1]
[1]University of São Paulo; [2]Imperial College London

How bacteria steal your candy: IgG glycan hydrolysis during streptococcal infection

Poster #: LB5 || Abstract #: 233

Andreas Naegeli, Christofer Karlsson, Eleni Bratanis, Adam Linder, Johan Malmström, Mattias Collin
Division of Infection Medicine, Department of Clinical Sciences, Lund University

Glycosylation alters oligomerization status of the human CEACAM1-IgV Domain

Poster #: LB6 || Abstract #: 234

James Prestegard, Kelley Moremen, You Zhuo, Kari Pederson, Jeong-Yeh Yang
University of Georgia

The role of L-fucose in cholera toxin intoxication of intestinal epithelial cells

Poster #: LB7 || Abstract #: 235

Amberlyn M. Wands[1], Akiko Fujita[1], Janet E. McCombs[1], Jakob Cervin[2], Andrea C. Rodriguez[1], Nicole Nischan[1], Ulf Yrlid[2], Jennifer J. Kohler[1]
[1]University of Texas Southwestern Medical Center; [2]University of Gothenburg

POSTER PROGRAM

Analysis of immunoglobulin Fc N-glycosylation for biopharmaceutical and clinical samples

Poster #: LB8 || Abstract #: 236

David Falck[1], Albert Bondt[1,2], Bas C. Jansen[1], Willem Jan R. Fokkink[3], Rosina Plomp[1], Simone Nicolardi[1], Markus Haberger[4], Dietmar Reusch[4], Radboud J.E.M. Dolhain[2], Bart C. Jacobs[3], Manfred Wuhrer[1]

[1]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands; [2]Department of Rheumatology, Erasmus University Medical Center, Rotterdam, The Netherlands; [3]Departments of Immunology and Neurology, Erasmus University Medical Center, Rotterdam, The Netherlands; [4]Pharma Biotech Development Penzberg, Roche Diagnostics GmbH, Penzberg, Germany

Synthesis of structurally defined heparin sulfate oligosaccharides

Poster #: LB9 || Abstract #: 237

Vijayakanth Pagadala

Glycan Therapeutics LLC

Clostridium Difficile Surface Polysaccharide-KLH Conjugate Vaccine Induced Th17-featured Adaptive Immune Responses in Mice

Poster #: LB10 || Abstract #: 238

Shuguang Bi

Stellar Biotechnology

Glycobiology helps unveil Trypanosoma membrane physiology

Poster #: LB11 || Abstract #: 239

Andres Lantos[1], Giannina Carlevaro[1], Betriz Araoz[2], Pablo Ruiz Diaz[1], Mariano Bossi[2], Hai Yu[3], Xi Chen[3], Carolyn Bertozzi[4], Juan Mucci[1], Oscar Campetella[1]

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Reactive Oxygen Species (ROS) Mediated De-sialylation on Glycoproteins and Cells

Poster #: LB12 || Abstract #: 240

Peng G. Wang, Maohui Wei, Tiehai Li, Jing Li

Department of Chemistry, Georgia State University

SESSION III: Glycan Binding Proteins, Glycosyltransferases, and Glycosidases-Structure and Function

Interaction of Meningococcal Group X N-Acetylglucosamine-1-Phosphotransferase with its Substrates

Poster #: LB14 || Abstract #: 241

Willie F. Vann[1], Ebony Cottman-Thomas[1], Gerd K. Wagner[2], Yi Chen[3], Xi Chen[3], Chao Cai[4], Robert J. Linhardt[4], Shonoi Ming[1]

[1]Laboratory of Bacterial Polysaccharides, FDA, Silver Spring, MD USA; [2]Department of Chemistry, King's College London, UK; [3]Department of Chemistry, University of California, Davis, CA USA; [4]Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute Troy, NY, USA

Interactions of the Cytokine Pleiotrophin with Glycosaminoglycans

Poster #: LB12 || Abstract #: 242

Xu Wang, Eathen O. Ryan, Di Shen

Arizona State University

A tumor-associated glycoform of MUC1 modulates the tumor microenvironment through engagment of Siglec 9

Poster #: LB13 || Abstract #: 243

Joy M. Burchell[1], Richard Beatson[1], Virginia Tajadura-Ortega[1], Gianfranco Picco[1], Daniela Achkova[1], Sandra Klausing[2], Thomas Noll[2], Joyce Taylor-Papadimitriou[1]

[1]King's College London; [2]University of Bielefeld

POSTER PROGRAM

Role of hepatic heparan sulfates in the regulation of hepcidin expression and iron homeostasis

Poster #: LB16 || Abstract #: 244

Maura Poli[1], Paola Ruzzenenti[1], Michela Asperti[1], Annamaria Naggi[2], Paolo Arosio[1]
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Critical role of galectin-3 in influenza virus infection by regulating the host immune responses

Poster #: LB17 || Abstract #: 245

Yu-Jung Chen[1], Sheng-Fan Wang[2], Huan-Yuan Chen[1,3], Fu-Tong Liu[1,3]
[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; [2]Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University , Kaohsiung, Taiwan; [3]Department of Dermatology, University of California Davis, CA

Cholera toxin binding influenced by mixed gangliosides: a binding cooperativity study using a high-throughput cell membrane array

Poster #: LB18 || Abstract #: 246

Hung-Jen Wu, Nolan C. Worstell, Pratik Krishnan, Joshua D. Weatherston
Texas A&M University

Combining 3D Structure with Glycan Array Data Provides Insight into the Origin of Glycan Specificity

Poster #: LB19 || Abstract #: 247

Robert J. Woods, Oliver C. Grant
University of Georgia

The GCNT2/Galectin-3 Axis Regulates Melanoma Malignancy

Poster #: LB20 || Abstract #: 248

Jenna Geddes Sweeney[1,2], Jennifer Liang[1], Nicholas Giovannone[1,2], Yoshihiko Tani[4], Lana Schaffer[5], Steven R. Head[5], Aristotelis Antonopoulos[3], Stuart M. Haslam[3], Hans R. Widlund[1], Charles J. Dimitroff[1,2]
[1]Brigham and Women's Hospital; [2]Harvard Medical School; [3]Imperial College London; [4]Japanese Red Cross Kinki Block Blood Center; [5]The Scripps Research Institute

Synthesis of LacdiNAc compounds using a bacterial glycosyltransferase

Poster #: LB21 || Abstract #: 249

Michel Gilbert, N. Martin Young, Melissa J. Schur
National Research Council Canada

In vivo tagging of non-cellulosic plant cell wall glycans using tagged CBM and scFv probes

Poster #: LB22 || Abstract #: 250

Michael G. Hahn, Fangfang Fu, Tiantian Zhang, Si Zhang, Utku Avci
University of Georgia

Polar enrichment of glycosyltransferases involved in the cell envelope biogenesis in Mycobacterium smegmatis

Poster #: LB23 || Abstract #: 251

Yasu S. Morita, Chu-Yuan Luo, Tsungda Hsu, Jennifer M. Hayashi
University of Massachusetts Amherst

Glycan ligands for targeting siglecs on immune cells

Poster #: LB24 || Abstract #: 252

Corwin Nycholat, Shiteng Duan, Ryan McBride, Anzhi Yao, James C. Paulson
The Scripps Research Institute La Jolla

GD3 identifies glioblastoma stem cells and GD3 synthase is a key driver for tumorigenicity

Poster #: LB25 || Abstract #: 253

Shih-Chi Yeh[1,2], Yi-Wei Lou[2], Pao-Yuan Wang[2,3], Michael Hsiao[2], Tsui-Ling Hsu[2], Chi-Huey Wong[2]
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POSTER PROGRAM

Galectin-3 Binding Protein and Galectin-1 Interaction in Breast Cancer Cell Aggregation and Metastasis

Poster #: LB26 || Abstract #: 254

Hui-Tzu Chang[1,2], Tzu-Wen Lin[1], Chein-Hung Chen[1], Chung-Hsuan Chen[1], Sheng-Wei Lin[3], Tsui-Ling Hsu[1], Chi-Huey Wong[1]

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Using ReSET and Sialosyl Iodide Glycosylation to Develop Chemical Probes to Study Sialic Acid (Neu5Ac) Biochemistry

Poster #: LB27 || Abstract #: 255

Simon S. Park, Jacquelyn Gervay-Hague

University of California, Davis

Using ReSET and Sialosyl Iodide Glycosylation to Develop Chemical Probes to Study Sialic Acid (Neu5Ac) Biochemistry

Poster #: LB27 || Abstract #: 255

Simon S. Park, Jacquelyn Gervay-Hague

University of California, Davis

SESSION IV: Glycomics and Glycoengineering

Functional Landscaping of the Cell Surface Glycome

Poster #: LB28 || Abstract #: 256

Dayoung Park[1], Narine Arabyan[1], Anupam Mitra[2], Bart C. Weimer[1], Emanual Maverakis[2], Carlito B. Lebrilla[1]

[1]University of California, Davis; [2]University of California, Davis School of Medicine

Cell surface N-glycans remodeled in vitro using commercial glycosidases

Poster #: LB29 || Abstract #: 257

Paula Magnelli[1], Alejandro Cagnoni [2], Luciano Morosi [2], Anabela Cutine [2], Amberlyn Wands [3], Jennifer Kohler [3], Karina Mariño [2]

[1]New England Biolabs; [2]IBYME. Buenos Aires, Argentina; [3]UT Southwestern Medical Center, Dallas, TX

Identifying the molecular determinants of N-linked glycosylation in Campylobacter jejuni

Poster #: LB30 || Abstract #: 258

Julie M. Silverman, Barbara Imperiali

Massachusetts Institute of Technology

Unraveling the complexity of glycosylation through systems biology

Poster #: LB31 || Abstract #: 259

Nathan E. Lewis

University of California, San Diego

pGlycoFilter: A tool to aid identification of plant N-linked glycans from mass spectrometry data

Poster #: LB32 || Abstract #: 260

Margaret R. Baker, Qing X. Li

University of Hawaii

Linkage-specific sialic acid derivatization for MALDI-TOF-MS profiling of IgG glycopeptides

Poster #: LB33 || Abstract #: 261

David Falck[1], Noortje de Haan[1], Karli R. Reiding[1], Markus Haberger[2], Dietmar Reusch[2], Manfred Wuhrer[1]

[1]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands; [2]Pharma Biotech Development Penzberg, Roche Diagnostics GmbH, Penzberg, Germany

POSTER PROGRAM

Lectin Microarray Analysis Reveals Impact of Microbicide Formulation on Glycosylation and Innate Immunity in Cervicovaginal Fluid

Poster #: LB34 || Abstract #: 262

Lara K. Mahal[1], Sujeethraj Koppolu[1], Linlin Wang[1], Charlene Dezzutti[2], Charles Isaacs[3], Bernard Moncla[2], Sharon Hillier[2], Lisa Rohan[1]
[1]New York University; [2]Magee Women's Research Institute; [3]New York State Institute for Basic Research

A miRNA Proxy Approach to Decoding the Glycome

Poster #: LB35 || Abstract #: 263

Lara K. Mahal, Tomasz Kurcon, Zhongyin Liu, Christopher Vaiana, Sujeethraj Koppolu, Brian Kasper
New York University

Unusual structural features of extracellular polysaccharides and glycoproteins in the hydrocarbon-producing alga Botryococcus braunii

Poster #: LB36 || Abstract #: 264

Mayumi Ishihara[1], Ian Black[1], Christian Heiss[1], Mehmet Tatli[2], Timothy P. Devarenne[2], Parastoo Azadi[1]
[1]Complex Carbohydrate Research Center, University of Georgia, GA; [2]Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX

High throughput antibody analysis using Rapid Deglycosylation, Direct Labeling, and Proteases

Poster #: LB37 || Abstract #: 265

Paula Magnelli, Elizabeth McLeod, Colleen McClung, Alicia Bielik, Ellen Guthrie
New England Biolabs, Ipswich, MA

Glycopeptide analogue of PSGL-1 binds to P-selectin with nanomolar affinity in vitro

Poster #: LB38 || Abstract #: 266

Ying Yu[1,3], Venkata Krishnamurthy[2], xuezheng Song[1], Yi Lasanajak[1], Elliot Chaikof[2], Richard D. Cummings[1,2]
[1]Biochemistry Department, Emory University School of Medicine; [2]Beth Israel Deaconess Medical Center and Harvard Medical School; [3]Perinatal Institute, Cincinnati Children's Medical Center

Isotope Targeted Glycoproteomics for Intact N- and O-Glycopeptide Discovery

Poster #: LB39 || Abstract #: 267

Christina M. Woo[1], Carolyn R. Bertozzi[1,2,3]
[1]Department of Chemistry, Stanford University, Stanford, CA 94305; [2]Department of Chemical and Systems Biology, Stanford University, Stanford, CA 94305; [3]Howard Hughes Medical Institute

SESSION V: The Many Functions of O-GlcNAc

A mutant O-GlcNAcase as a probe to reveal global dynamics of the O-GlcNAc proteome during Drosophila embryonic development

Poster #: LB40 || Abstract #: 268

Nithya Selvan[1], Daniel Mariappa[1], Vladimir S. Borodkin[1], Jana Alonso[1], Andrew T. Ferenbach[1], Claire Shepherd[2], Iva Hopkins Navratilova[2], David Campbell[1], Daan MF van Aalten[1,3]
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Physiological Consequences of Loss of OGT Dependent O-GlcNAcylation in Hepatocytes

Poster #: LB41 || Abstract #: 269

Krista Kaasik, Robert Chalkley, Alma Burlingame
University of California San Francisco

Disruption of Brain O-GlcNAc cycling induces OTX2-dependent developmental delay

Poster #: LB41 || Abstract #: 269

Stephanie Olivier-Van Stichelen, Peng Wang, Johua Ohde, John Hanover
National institute of Health

POSTER PROGRAM

Analysis of the biological roles of extracellular O-GlcNAc using EOGT-deficient mice

Poster #: LB43 || Abstract #: 271

Mitsutaka Ogawa[1], Paweł Bieniasz-Krzywiec[1], Hirokazu Yagi[2], Koichi Kato[2,3], Jiro Usukura[4], Koichi Furukawa[1], Tetsuya Okajima[1]

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O-GlcNAc cycling plays a crucial role in regulation of adult reproductive diapause in C. elegans

Poster #: LB44 || Abstract #: 272

Moriah Eustice, John Hanover

NIDDK

OGT Regulates Vascular Cell Proliferation and Glucose Utilization in Idiopathic Pulmonary Arterial Hypertension

Poster #: LB45 || Abstract #: 273

Jarrod W. Barnes[1,3], Liping Tian[1,3], Suzy A. Comhair[1,3], Raed A. Dweik[1,2,3]

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Identification and label-free quantification of O-GlcNAc sites in activated human T cells

Poster #: LB46 || Abstract #: 274

Peder J. Lund[1,9], Christina M. Woo[2,9], Sharon J. Pitteri[3,4], Carolyn R. Bertozzi[2,5,6], Mark M. Davis[7,8,6]

[1]Interdepartmental Program in Immunology, Stanford University; [2]Department of Chemistry, Stanford University; [3]Department of Radiology, Stanford University; [4]Canary Center at Stanford for Cancer Early Detection; [5]Department of Chemical and Systems Biology, Stanford University; [6]Howard Hughes Medical Institute; [7]Department of Microbiology and Immunology, Stanford University; [8]Institute for Immunity, Transplantation, and Infection; [9]Co-first authors

An Integrated Solution for High-throughput, User-friendly Glycoanalysis Using Rapid Separation by Capillary Electrophoresis

Poster #: LB47 || Abstract #: 275

Zoltan Szabo, Michael Kimzey, Shirley Ng, Alexander Gyenes, Adele Taylor, Aled Jones, Justin Hyche, Ted Haxo, Sergey Vlasenko

ProZyme, Inc.

SESSION VII: Applied Glycobiology

Structural snapshots of Notch xylosylation support an SNi-like retaining mechanism

Poster #: LB48 || Abstract #: 276

Hongjun Yu[1], Megumi Takeuchi[2], Jamie LeBarron[2], Joshua Kantharia[2], Erwin London[2], Hans Bakker[3], Robert S. Haltiwanger[2], Hideyuki Takeuchi[2], Huilin Li[1,2]

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Another function of N-linked glycosylation: preventing deamidation in glycoproteins and glycopeptides

Poster #: LB49 || Abstract #: 277

Peng G. Wang, Jingyao Qu, Hailiang Zhu

Department of Chemistry, Georgia State University

Homogeneous Heparan Sulfate Oligomers for NMR Studies

Poster #: LB50 || Abstract #: 278

Kari Pederson[1], Rachel Vecchione[1], Kelley McCloy[2], Shuo Wang[1], Kelley W. Moremen[1], James H. Prestegard[1]

[1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602; [2]Department of Chemistry, Wittenberg University, Springfield, OH 45501

POSTER PROGRAM

Rapid-throughput glycomics to discover the role of human milk oligosaccharides in infant health

Poster #: LB51 || Abstract #: 279

Jasmine C. C. Davis[1], Sarah M. Totten[1], Lauren D. Wu[1], Evan A. Parker[1], Angela M. Zivkovic[2], Carlito B. Lebrilla[1]
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SESSION VIII: Glycan Expression during Development and Differentiation

ppGalNac-T13 was Required for Stabilizing Mucin-Type O-glycoprotein Podoplanin during P19 Cell Neuronal Differentiation

Poster #: LB52 || Abstract #: 280

Yan Zhang, Yingjiao Xu, Aidong Shan, Lei Zhang, Wenjie Pang
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Development of a rapid HILIC-UPLC method for glycan analysis of recombinant proteins

Poster #: LB53 || Abstract #: 281

Yunli Hu, Ruth Frenkel, Zoran Sosic
Biogen

A versatile, high-performance microarray displaying site-specifically glycosylated mucin glycopeptides to detect antibodies directed to disease-relevant conformational epitopes

Poster #: LB54 || Abstract #: 282

Yoshiaki Miura[1], Taichi Aihara[1], Hiroshi Hinou[2], Shun Hayakawa[2], Shobith Rangappa[2], Gerard Artigas[2], Kazuhiro Nouso[4], Yasuhiro Miyake[4], Kazuhide Yamamoto[4], Shin-Ichiro Nishimura[2,3]
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SESSION IX: Diseases and Disorders of Glycosylation

Aberrant O-glycosylation affects the transcriptional response downstream of MUC1 and EGFR in breast cancer

Poster #: LB55 || Abstract #: 283

Virginia Tajadura, Gennaro Gambardella, Joyce Taylor-Papadimitriou , Francesca Ciccarelli , Joy Burchell
King's College London

Solid-phase extraction of N-linked glycans and glycosite-containing peptides (NGAG) for comprehensive characterization of glycoproteins

Poster #: LB56 || Abstract #: 284

Shisheng Sun, Punit Shah, Shadi Toghi Eshghi, Weiming Yang, Namita Trikannad, Shuang Yang, Lijun Chen, Paul Aiyetan, Naseruddin Höti, Zhen Zhang, Daniel W. Chan, Hui Zhang
[1]S-BIO, Vaupell Holdings Inc.; [2]Faculty of Advanced Life Science, Hokkaido University; [3]Medicinal Department of Pathology, Johns Hopkins University, Baltimore, Maryland 21287, USA

GPQuest: A Software Tool for Glycoproteomics Analysis of Complex Biological Samples

Poster #: LB57 || Abstract #: 285

Shadi Eshghi, Weiming Yang, Punit Shah, Shisheng Sun, Xingde Li, Hui Zhang
Johns Hopkins Medical Institutions

POSTER PROGRAM

Disruption of Golgi morphology and altered protein glycosylation in PLA2G6-associated neurodegeneration

Poster #: LB58 || Abstract #: 286

Mariska Davids[1,2], Megan S. Kane[1,2], Miao He[3,4], Lynne A. Wolfe[1,2], Xueli Li[3,4], Mohd A. Raihan[3,4], Katherine R. Chao[1,2], William P. Bone[1,2], Cornelius F. Boerkoel[5], William A. Gahl[1,2], Camilo Toro[1,2]
[1]NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, NIH, Bethesda, MD; [2]Office of the Clinical Director, NHGRI, National Institutes of Health, Bethesda, MD; [3]Department of Pathology and Laboratory of Medicine, University of Pennsylvania, Philadelphia, PA; [4]The Michael J Palmieri Metabolic Laboratory, Children's Hospital of Philadelphia, Philadelphia, PA; [5]University of British Columbia, Vancouver, Canada

An agnostic screen of protein glycosylation in the attempt to diagnose rare genetic disorders and unravel mechanism of disease

Poster #: LB59 || Abstract #: 287

Megan S. Kane[1,2], Mariska Davids[1,2], Miao He[3,4], Cornelius F. Boerkoel[5], William A. Gahl[1,2]
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Toxoplasma gondii microneme proteins 1 and 4 recognize N-glycans of TLRs: (1) TgMIC1 and TgMIC4 interact with TLR2 N-glycans

Poster #: LB60 || Abstract #: 288

Flávia Costa Mendonça Natividade[1], Ana Cláudia Paiva Alegre Maller[1], Aline Sardinha da Silva[1], Nicholas Gay[2], Maria Cristina Roque Barreira[1]
[1]Laboratório de Imunoquímica e Glicobiologia, Departamento de Biologia Celular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14049900, Ribeirão Preto; [2]Departament of Biochemistry, Cambridge University, 80 Tennis Court Road Cambridge CB2 1GA, United Kingdom

Toxoplasma gondii microneme proteins 1 and 4 recognize N-glycans of TLRs: (2) the interaction triggers the initial immune response to the protozoan

Poster #: LB61 || Abstract #: 289

Aline Sardinha-Silva[1], Flávia Costa Mendonça-Natividade[1], Camila Figueiredo Pinzan[1], Carla Duque Lopes[1], André Vieira Zorzetto-Fernandes[1], Diego Luís Costa[2], Damien Jacot[3], Alan Sher[2], Dominique Soldati-Favre[3], Dragana Jankovic[2], Maria Cristina Roque Barreira[1]
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Targeting Human Cancer by a Glycosaminoglycan Binding Malaria Protein

Poster #: LB62 || Abstract #: 290

Thomas M Clausen[1,2,3,4#] Ali Salanti[1,*#], Mette Ø Agerbæk[1,2,3], Nader Al Nakouzi[2,3,] Htoo Z Oo[2,3] , Jamie R Rich[5,6], Yang Mao[7], Marina A Pereira[1], Joseph Zaia[7], Thor G Theander[1], Poul H Sorensen[3] and Mads Daugaard[2,3,5] [#]Contributed equally to the work
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MODULATION OF THE INNATE IMMUNE RESPONSES TO PATHOGENIC ESCHERICHIA COLI BY PAIRED SIGLEC RECEPTORS IN HUMANS ANDIN MICE

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Paired receptors in the immune system are membrane proteins containing similar extracellular regions but exhibiting opposite potential for signaling, with one member sending inhibitory signals and the other activating. Typically, the inhibitory receptors bind to self-antigens to limit cellular activation. By contrast, the functions of the activating counterparts are often less defined. Two sets of paired receptors have been identified in the sialic acid-binding Siglec family in humans, with unexplained polymorphic pseudogenization of the activating members. Siglec-5/14 were recently shown to bi-directionally modulate inflammatory responses to the sialylated bacterial pathogen group B *Streptococcus*. Here, we report that Siglec-11 and Siglec-16 are paired receptors expressed on tissue macrophages and on microglia in humans. We provide evidence that pathogenic *Escherichia coli* K1 can engage the two Siglecs on macrophages through its polysialic acid capsular polysaccharide. However, whereas binding of bacteria to the immune cells expressing Siglec-11 favors bacterial survival, the presence of Siglec-16 increases bacterial killing. To further explore the significance of human paired Siglec receptors in a robust in vivo model, we generated a knock-in mouse line expressing a chimeric receptor that includes the extracellular part of Siglec-E and the transmembrane segment of Siglec-16. The engineered Siglec-E16 protein showed a tissue expression pattern similar to Siglec-E, but rather than promoting inhibitory signaling, it recruited DAP12 and increased pro-inflammatory cytokines expression in macrophages upon LPS stimulation. Following intravenous *E. coli* challenge, Siglec-E16 mice produced more pro-inflammatory cytokines, restricted bacterial proliferation in blood and limited bacterial dissemination to the spleen and liver. We conclude that paired Siglec receptors modulate host innate immune and inflammatory responses *in vivo* upon bacterial infection. These data also support the hypothesis that activating receptors have evolved to counterbalance pathogens that target the inhibitory pathway by sialic acid mimicry. Lastly, the Siglec-E16 mouse line may provide a valuable tool to test the hypothesis that activating Siglecs are undergoing elimination in humans, because they induce excessive inflammatory responses that are deleterious for the host.

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OVERLAPPING SUBSTRATE SPECIFICITY OF NUCLEOTIDE SUGAR TRANSPORTERS IN THE FUNGAL PATHOGEN, ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is an opportunistic fungal pathogen that is the primary cause of the systemic fungal infection, invasive aspergillosis. Current therapies for Aspergillus infections are inadequate and there is growing drug resistance. The fungal cell wall is a network of complex carbohydrates and it is the primary target for the development of antifungals due to its absence on mammalian cells. Presentation of sugars on the cell surface are mediated by nucleotide sugar transporters (NSTs), proteins that transport activated sugars from the cytosol into the ER/ Golgi lumen to be added to glycosidic chains. Four A. fumigatus NSTs (AfNSTs) were cloned and characterized, and two A. fumigatus NST knockout mutants were created. Assays using Golgi-enriched fractions from yeast overexpressing Afnst genes revealed that both AfNST1 and AfNST5 transported UDP-[14C]-Gal. Saturation transfer difference (STD)-NMR analyses revealed that several UDP-containing sugars were able to bind to the AfNSTs, but the combination of nucleotide sugars was not identical. Epitope maps generated from STD-NMR data revealed key contact points of the nucleotide sugars to the AfNSTs. This is the first demonstration of multi-substrate NSTs in fungi and the first identification of UDP-Gal transporters in filamentous fungi. The A. fumigatus knockout (KO) mutants of AfNST1 and AfNST5 showed significant differences in growth rate and morphology compared to wild type on media supplemented with the polysaccharide-binding dye, Congo Red. Flow cytometry analyses revealed different levels of galactose and Neu5Ac on mutant spores compared to wild type. In conclusion, although both AfNST1 and AfNST5are UDP-Gal transporters, substrate differences may explain the observed differences in morphology under conditions of cell wall stress. Compensatory pathways demonstrate redundancy in fungal carbohydrate metabolism that has implications for the development of antifungal agents targeting cell wall glycans.

Keywords: fungal pathogen, invasive aspergillosis, UDP-sugar, nucleotide sugar transporters, Golgi

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C ELEGANS STRAINS DEFICIENT IN O-GLYCOSYLATION SHOW ALTERED TOLERANCES TO A RANGE OF BACTERIAL PATHOGENS: GLYCOSYLATION IMPACT ON INNATE IMMUNITY

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Caenorhabditis elegans generates pathogen resistance primarily through innate immune system response. The bus mutants (bacterial unswollen), isolated by their altered response to the nematode pathogen Microbacterium nematophilum, are known to have altered susceptibility to a number of additional pathogens including Yersinia pestis and Yersinia pseudotuberculosis. Here we extend that list to Staphylococcus aureus. The bus mutants fall into two categories which include, those with loss of function in innate immune system components and, those with loss direct loss of function in glycosylation pathways or their putative downstream products. Previously we studied reference strains of three mutants from the later class, bus-2, bus-4 and bus-17. In each we found distinct changes in O-glycan structure and O-glycoprotein distribution. While each of these harbors a defect in a separate putative glycosyltransferase gene, it was not clear if the glycosylation defects were directly causative of the altered pathogen resistance such as the loss of a receptor for bacterial infection. Here we further investigate the resistance phenotype of these three mutants at the gene expression level. We have found common shifts in expression in all three mutants across four distinct areas. These include: 1) N- and O-glycosylation; 2) innate immune response; 3) protein folding editing control, and; 4) cuticle structure. Changes in O-glycosylation interact with these four classes of gene expression that results in changes in resistance that likely goes beyond changes in cell surface glycosylation or glycosylation of secreted glycoproteins. In this work we demonstrate that bus-2, bus-4 and bus-17 mutants are resistant to infection by Staphylococcus aureus. Increased gene expression in innate immune system components previously seen in response to gram positive bacteria was seen across these strains in un-infected conditions and a subset of these genes are further upregulated upon infection with S. aureus. The overall shift in expression in these four areas leads to better fitness to respond to pathogenic challenge to S. aureus and likely other pathogens.

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VACCINATION WITH RECOMBINANT MICRONEME LECTINS CONFERS PROTECTION AGAINST EXPERIMENTAL TOXOPLASMOSIS IN MICE

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Toxoplasmosis, a zoonotic disease caused by Toxoplasma gondii, is an importante public health problem and veterinary concern.Toxoplasma gondii actively infect host cells through a dependent process of lectins release, called microneme proteins (MIC), from intracellular organelles. TgMIC1, TgMIC4 and TgMIC6 assembly a complex on T. gondii surface enabling the parasite to bind to host cells via carbohydrate recognition, since TgMIC1 binds to terminal sialic acid and TgMIC4 to terminal galactose.Although there is no vaccine for humantoxoplasmosis, many attempts have been made to develop one. Promising vaccinecandidates utilize proteins, or their genes, from microneme organelle of T. gondii that are involved in the initial stages of host cell invasion by the parasite.In the presentstudy, we used different recombinant microneme proteins (TgMIC1, TgMIC4, orTgMIC6) or combinations of these proteins (TgMIC1-4 and TgMIC1-4-6) to evaluatethe immune response and protection against experimental toxoplasmosis in C57BL/6mice. Vaccination with recombinant TgMIC1, TgMIC4, or TgMIC6 alone conferredpartial protection, as demonstrated by reduced brain cyst burden and mortality ratesafter challenge. Immunization with TgMIC1-4 or TgMIC1-4-6 vaccines provided themost effective protection, since 70% and 80% of mice, respectively, survived to theacute phase of infection. In addition, these vaccinated mice, in comparison to nonvaccinatedones, showed reduced parasite burden by 59% and 68%, respectively. Theprotective effect was related to the cellular and humoral immune responses induced byvaccination and included the release of Th1 cytokines IFN-γ and IL-12, antigenstimulatedspleen cell proliferation, and production of antigen-specific serumantibodies. Our results demonstrate that microneme proteins are potential vaccinesagainst T. gondii, since their inoculation prevents or decreases the deleterious effectsof the infection.

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HOW BACTERIA STEAL YOUR CANDY: IGG GLYCAN HYDROLYSIS DURING STREPTOCOCCAL INFECTION

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Streptococcus pyogenes (group A streptococcus) is an important human pathogen responsible for a wide variety of diseases from mild pharyngitis to live-threatening invasive infections and postinfectious immune-mediated disorders. It secretes EndoS, an endoglycosidase able to cleave the conserved *N*-glycan on human immunoglobulin G (IgG) antibodies. This leads to inactivation of IgG effector functions such as complement activation or antibody-mediated phagocytosis. While proposed as a potential virulence factor, the importance of EndoS during an infection has so far not been elucidated.

We have developed a targeted mass spectrometry approach to quantitatively assess the degree of IgG glycan hydrolysis directly from patient samples. With this, we have analyzed samples from both invasive as well as local infections. In the case of invasive streptococcal infections, the degree of IgG glycan hydrolysis correlates with disease severity, indicating a role for EndoS in streptococcal virulence. This study offers new insights into streptococcal pathogenesis and might therefore be relevant for the treatment of severe streptococcal infections.

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GLYCOSYLATION ALTERS OLIGOMERIZATION STATUS OF THE HUMAN CEACAM1-IGV DOMAIN

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Human carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a cell-surface signaling molecule involved in cell adhesion, proliferation and immune response. It is also implicated in cancer angiogenesis, progression and metastasis. This diverse set of roles likely arises as a result of the numerous homophilic and heterophilic interactions that CEACAM1 can have with itself and other members of the CEACAM superfamily. Its N-terminal Ig-like variable (IgV) domain has been suggested to be a principal player in these interactions, facilitating cis and trans homo-dimer formation, as well as complex formation with other molecules. The IgV domain crystal structure, which used E. coli expressed material that lacks glycosylation, shows a dimer interface that obscures a glycosylation site, while other studies using mutagenesis and electron microscopy suggest dimer formation may also be mediated by a non-glycosylated surface on the opposite side of the molecule. Here, we use NMR cross correlation measurements and residual dipolar coupling measurements to examine the effect of glycosylation on CEACAM1-IgV domain oligomerization status. Both a non-glycosylated form expressed in E. coli and a glycosylated form expressed in HEK293 GnT1- cells and trimmed to a single GlcNAc residue, were examined. Our findings demonstrate that IgV with a single N-linked GlcNAc at all glycosylation sites inhibits dimer formation of any type, whereas formation of a dimer similar in structure to that found in the crystal structure occurs in solution when glycosylation is absent. Future studies will be directed at the role additional domains and more complex glycosylation may play in native dimer formation.

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THE ROLE OF L-FUCOSE IN CHOLERA TOXIN INTOXICATION OF INTESTINAL EPITHELIAL CELLS

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Cholera toxin (CT) utilizes the thick glycan coating on the plasma membrane of the intestinal epithelium to invade host tissue, and its retrograde transport to the cytosol results in the massive loss of fluids and electrolytes associated with severe dehydration. It is widely accepted that the mono-sialoganglioside GM1 is cholera toxin's sole receptor to initiate this process. However, the application of a photocrosslinking strategy that involves metabolic incorporation of diazirine-modified sialic acid residues (SiaDAz) into either T84 or Colo205 intestinal epithelial cell lines has resulted in the covalent capture/detection of complexes formed between cholera toxin subunit B (CTB) and its sialylated glycoprotein binding partners. In addition to sialylation, co-culture of these cells with an increasing amount of peracetylated 2-fluorofucose (2F-Fuc), a metabolic inhibitor of fucosylation, can inhibit SiaDAz photocrosslinking of CTB to its glycoprotein binding partners; therefore indicating that these glycoproteins of interest most likely possess a fucosylated glycan as the binding epitope. To this end, competition binding assays that include various naturally occurring monosaccharaides reveal that while L-fucose contributes significantly to CTB binding to intestinal epithelial cell lines that express very low levels of GM1, it has no effect on the ability of the toxin to bind to a T lymphocyte cell line that expresses a much higher level of GM1. Furthermore, 2F-Fuc decreases endocytic uptake of CTB into T84 cells, and decreases stimulation of cAMP accumulation in T84 monolayers upon induction with the holotoxin. Future work entails the elucidation of the fucosylated glycan(s) that mediate cell surface binding, as well as the residues within the CTB binding site that are being targeted, in the hopes that biomimetics can be developed for the specific blocking of CT targeting to the intestinal epithelium.

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ANALYSIS OF IMMUNOGLOBULIN FC N-GLYCOSYLATION FOR BIOPHARMACEUTICAL AND CLINICAL SAMPLES

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Glycosylation has a large functional impact on immunoglobulins (Igs). For example, the conserved *N*-glycosylation site in the Fc part of IgG influences binding to receptors, such as the Fcγ receptor family. Consequently, glycosylation is important for antibody mediated complement activation and antibody dependent cellular cytotoxicity. Furthermore, Fc *N*-glycosylation affects stability of the antibody higher order structure, as seen in temperature stability and susceptibility to proteases. We present a recent detailed investigation of the influence of glycosylation on tryptic cleavage of a monoclonal antibody and donor-derived biopharmaceutical formulations (intravenous IgG; IVIG). Trypsin showed a preference for the cleavage of high mannose, hybrid-type, bisected and alpha2-3-linked sialic acid containing glycans, especially when the antibody had not been denatured prior to incubation.

IVIG is widely applied for the treatment of both immune deficiencies, where it acts pro-inflammatory, and autoimmune diseases, where it acts anti-inflammatory. However, treatment outcomes can vary and the challenge to define such a complex product is immense. Variation in Fc *N*-glycosylation, which is treated as a critical quality attribute in monoclonal antibodies, but not in IVIG, was suspected to be a possible contributor to observed clinical effects. Therefore, we assessed the Fc *N*-glycosylation of in total 154 batches of seven different IVIG formulations available in Western-Europe. Fortunately, though we observed some interesting differences, both intra- and inter-product variation were minor.

The IgG-Fc glycosylation has been suggested to be a major factor in either pro- and anti-inflammatory functioning of immunoglobulins. For example, in autoimmune diseases, where antibodies recognize 'self'-antigens and thereby induce inflammation, lower levels of galactosylation are widely recognized. We have studied this phenomenon in the context of rheumatoid arthritis (RA) and pregnancy. Approximately fifty percent of the RA patients are known to have lower disease activity, reflecting lower inflammation, during pregnancy. Indeed, we have been able to positively correlate galactosylation of IgG-Fc with disease activity, which was independent of sialylation. Furthermore, the pregnancy associated increase of galactosylation is more pronounced in patients that do improve compared to the so-called non-responders. Similar observations were made on IgA1 *N*-glycopeptides, although differences between RA and healthy individuals were restricted to only one of the two sites.

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SYNTHESIS OF STRUCTURALLY DEFINED HEPARIN SULFATE OLIGOSACCHARIDES

Vijayakanth Pagadala

Glycan Therapeutics LLC

Glycan Therapeutics is a small biotech company pioneering in the synthesis of structurally defined heparan sulfate and heparin oligosaccharides for glycobiology researchers. Using the chemoenzymatic technology Glycan can synthesize heparan sulfate oligosaccharides with unprecedented structural complexity. Holding an exclusive license for the chemoenzymatic synthetic method developed by Professor Jian Liu from the University of North Carolina, Glycan has unmatched potential to make diverse heparan sulfate compounds. The company is now offering a catalogue of 24 homogeneous heparan sulfate oligosaccharides from 4-mers to 9-mers with a purity of greater than 98%. Glycan Therapeutics also offers custom synthesis of heparan sulfate oligosaccharides with different sizes, sulfation, and purity up to 18-mers. Customized chemical modification of oligosaccharides with different tags is also available.

Please visit our booth to ***sweeten and charge your heparan sulfate needs***. Limited free samples of structurally defined heparan sulfate oligosaccharide available at the booth.

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CLOSTRIDIUM DIFFICILE SURFACE POLYSACCHARIDE-KLH CONJUGATE VACCINE INDUCED TH17-FEATURED ADAPTIVE IMMUNE RESPONSES IN MICE

Shuguang Bi

Stellar Biotechnology

Clostridium difficile is a Gram-positive, anaerobic, spore-forming, toxin-producing bacterium. It is the leading cause of antibiotics associated diarrhea. C. difficile infection related morality and mortality has been increasing fast in the recent decades. As C. difficile is closely related to disturbed gut microbial flora caused by over usage of antibiotics, vaccines are being developed as an antibiotic-independent therapy. Toxin-based vaccine developed so far can reduce symptoms but cannot prevent recurrent infection, therefore, C. difficile surface layer proteins and various cell surface proteins are being targeted for blocking spore adhesion and vegetative bacteria colonization to prevent recurrent infection. As most of these proteins are heavily glycosylated, at Stellar Biotech, we explored the potential of C. difficile cell surface polysaccharides as vaccine candidate. We vaccinated C57BL/6 mice with whole lysate from non-virulent strain (ATCC 43255) and hyper virulent strains (ribotypes 106 and 027) as well as with vaccine prepared by conjugating 43255-derived polysaccharides to keyhole limpet hemocyanin (KLH). CD4+ T cells from the vaccinated mice were reacted in vitro with mitomycin-treated dendritic cells that were pretreated with 43255-derived polysaccharides, polysaccharide-BSA conjugate, and KLH. Cytokines released from the stimulated CD4+ T cells were profiled. All major Th1, Th2 and Th17 cytokines were studied. For the first time to our knowledge, we report here that (1) whole lysate from C. difficile induced strain-specific T cell-dependent immune responses; (2) Th17 responses were the dominant T cell responses; (3) polysaccharide-KLH conjugate, instead of polysaccharides alone, induced the generation of Th17 memory cells; (4) Polysaccharides from different strains are different, as evidenced by the fact that the vaccines prepared with non-virulent strain 43255 could not protect infection caused by hyper virulent strains 106 and 027.

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GLYCOBIOLOGY HELPS UNVEIL TRYPANOSOMA MEMBRANE PHYSIOLOGY

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Sialic acid glycobiology is crucial for the infective trypomastigote stage of *Trypanosoma cruzi*, the etiological agent of Chagas disease. Interestingly, *T. cruzi* is unable to synthesize sialic acids *de novo*. To tackle this issue it expresses the *trans*-sialidase (TS), a modified sialidase that catalyzes the transfer of sialyl residues between glycoconjugates. Mucins are a family of heavily *O*-glycosylated proteins that are major constituents of the trypomastigote surface and the main acceptors of sialic acid. The sialylation of mucins allows the parasite to avoid lysis by serum factors and to interact with the host cell. To study the biology of recently acquired sialic acid residues we adopted the unnatural sugar approach as bioorthogonal chemical reporters. Both mucins and TS are GPI-anchored proteins and substrate and enzyme in the sialic acid interplay. However it was surprising to find that they are separated in the trypomastigote membrane and contained in different and highly stable membrane microdomains. Mucins were included in lipid rafts whereas TS was not. Given their spatial separation, membrane bound TS is unable to sialylate mucins, a role played by the shed TS instead. We purified lipid rafts and determined its protein constituents by mass spectrometry. Lipid rafts proteins were also found in domains and were mainly associated to the flagellum. Finally we found that TS is shed only in microvesicles instead of as a fully soluble form as would result from an endogenous PI-PLC activity, which is not actually present in non-differentiating trypomastigotes.

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REACTIVE OXYGEN SPECIES (ROS) MEDIATED DE-SIALYLATION ON GLYCOPROTEINS AND CELLS

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Sialic acids are a diverse family of monosaccharides widely expressed on all cell surfaces of vertebrates, and play important roles in many physiological and pathological processes including cell signaling, bacterial and viral infection, and tumor metastasis. Desialylation, removal of sialic acids, is an essential part of sialic acid metabolism and is of paramount importance in the life process. Dysregulation of desialylation is highly associated with the pathogenesis of diabetes, cardiovascular diseases and various cancers. The mainstream idea attributes desialylation to the sialidases. However, more and more emerging evidences support the existence of non-sialidase desialylation in the life process. Recently, we evaluated the chemical bases of non-sialidase desialylation by electrolysis and reactive oxygen species (ROS) mediated desialylation of various sialic acid conjugates (Neu5Ac, Neu5Gc and KDN conjugated oligosaccharides, glycopeptides, glycoproteins and glycolipids). In both electrochemical desialylation and ROS mediated desialylation, α-2,3-linked sialic acids were easier to be desialylated than α-2,6-linked sialic acids. In electrochemical desialylation, conjugated sialic acids were removed via oxidative decarboxylation and only 4-(acetylamino)-2,4-dideoxy-D-glycero-D-galacto-octonic acid (ADOA) was provided. In ROS mediated desialylation, conjugated sialic acids were removed by ROS via radical mechanism to give free sialic acids, then released free sialic acids were further oxidized to ADOA. These results indicated that under oxidative stress, non-sialidase desialylation may occur in the life process.

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INTERACTION OF MENINGOCOCCAL GROUP X N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE WITH ITS SUBSTRATES

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Neisseria meningitidis is a major cause of bacterial meningitis in the meningitis belt of Sub-Saharan Africa. *N. meningitidis* serogroup X, a serogroup for which a vaccine does not exist, is emerging with increasing prevalence in the developing world. The serogroup X capsular polysaccharide is a homopolymer of (α1-4)-linked *N*-acetyl-D-glucosamine -1-phosphate. We have recently shown that the formation of this polysaccharide is catalyzed by an N-acetylglucosamine-1-phosphotransferase, encoded by the gene csxA, formerly named xcbA. The csxA gene product bears significant homology with the mammalian lyosomal N-acetylglucosamine-1-phosphotransferase. The csxA gene product catalyzes the transfer of a GlcNAc-1-phosphate from sugar-nucleotide UDP-GlcNAc to the 4-hydroxyl of a terminal GlcNAc-1-phosphate on the growing polysaccharide chain. We have explored the interaction of this enzyme with substrate analogs of the sugar nucleotide donor, UDP-GlcNAc, modified on either the uracil or the sugar moiety. We tested these analogs as substrates and inhibitors of the GlcNAc-1-phosphotransferase reaction. Our results suggest that bulky groups on the 5 position of the uracil interfere with catalysis. We have also shown that the 4-hydroxyl and the 2-acetamido groups of the GlcNAc moiety influence the interaction of the sugar nucleotide donor with the enzyme.

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INTERACTIONS OF THE CYTOKINE PLEIOTROPHIN WITH GLYCOSAMINOGLYCANS

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Pleiotrophin (PTN) is a glycosaminoglycan-binding cytokine involved in stimulating mitogenesis and angiogenesis. PTN is crucial in neural development and tissue regeneration, but is also consistently overexpressed in cancer cells. Although PTN is known to bind a number of different receptors, its interactions with proteoglycan-based receptors such as N-syndecan or receptor-type protein tyrosine phosphatase zeta (PTPRZ) are vital to its activities. However, it is unclear how different domains of PTN regulate its activity, nor is there high resolution structural information on these complexes. We have determined the structure of PTN and conducted extensive structural analysis of PTN's interactions with both heparin and chondroitin sulfate (CS). Our data show that, although the unstructured C-terminal tail is not required for binding highly sulfated GAGs such as heparin or CS-E, it is crucial to maintaining stable interactions with CS-A, thus explaining why truncated PTN missing the tail cannot signal through the CS proteoglycan PTPRZ. Using paramagnetically labeled GAG ligands and proteins, we also studied the interactions of CS and heparin ligands with PTN at high resolution. Our data showed that basic amino acid cluster 2 (K68, K91 and R92) and residues in the hinge segment of PTN are the most critical GAG-binding site in PTN, and the interactions of PTN with GAGs place the reducing end of the ligand in close contact with the hinge and cluster 2. We discovered that PTN oligomerization is transient and weak in the absence of GAGs. However, both heparin and CS-E oligosaccharides are capable of inducing strong oligomerization of PTN. By studying the separated and paramagnetically-labeled domains, we ascertained that GAG ligands promote homodimerzation of the C-terminal thrombospondin type-1 repeat (TSR) domain (CTD) but not the N-terminal TSR domain (NTD). Furthermore, heterodimerzation of CTD and NTD is also promoted by GAG ligands. However, the orientation of interaction between the domains is heterogeneous as no single binding orientation is consistent with all paramagnetic data.

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A TUMOR-ASSOCIATED GLYCOFORM OF MUC1 MODULATES THE TUMOR MICROENVIRONMENT THROUGH ENGAGMENT OF SIGLEC 9

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The tumor microenvironment is populated by host cells, which have been 'educated' to support tumour growth, metastasis and an immunosuppressive environment. For cancer cells to remodel their immune microenvironment they need to acquire changes that result in the recruitment of monocytes and macrophages and the repolarisation of resident macrophages to become tumor-promoting cells. Changes in the composition of glycans in glycoproteins are common events in cancer making it a hallmark of the disease, and as mucins carry large numbers of O-linked glycans it is particularly evident in this class of molecules. Aberrant glycosylation in breast and other cancers results in the O-linked glycans carried by the mucin, MUC1, being mainly short core1 glycans (Gal beta1-3GalNAc) that are often sialylated. Siglec 9 is a sialic acid binding lectin expressed on certain cells of the immune system and can negatively regulate the immune and inflammatory response through its two ITIM motifs. However the functional effects of binding to a specific natural ligand is still to be determined. Through the engagement of Siglec 9, we show that MUC1-ST (MUC1 carrying sialylated core 1) can educate myeloid cells to release factors that are associated with microenvironment determination and disease progression, and to induce the differentiation into alternatively activated phenotypes including tumor associated macrophages (TAMs) exemplified by the expression of CD206 and IDO. Unexpectedly we show that MUC1-ST binding to Siglec 9 does not activate SHP1/2 but induces a calcium flux leading to ERK and MAPK activation. This work sheds light on MUC1-ST's critical role in tumour biology, identifying its role in modulating the microenvironment.

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ROLE OF HEPATIC HEPARAN SULFATES IN THE REGULATION OF HEPCIDIN EXPRESSION AND IRON HOMEOSTASIS

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Liver is rich in highly sulfated heparan sulfates (HS), which have been found to have an important role in the clearance of plasma lipoprotein. We propose that they have a role also in iron metabolism and the production of hepcidin, a hormone peptide that regulates the systemic iron availability controlling the absorption of the dietary iron and the recycle of hemoglobin iron. Hepcidin expression occurs mainly in the liver, where it is regulated mostly by the BMP6/SMAD1/5/8 pathway. We have recently found that heparins, even the non-anticoagulant ones, are strong inhibitors of hepcidin expression in hepatic cells and in mouse liver and that their activity is stronger when highly sulfated and with molecular weight >7 kD. Part of this activity is attributed to heparin capacity to interfere/sequester the BMP6. To clarify this, we started analyzing the three putative heparin binding domains (HBD) identified in human BMP6. The synthetic peptides corresponding to the N-terminal one showed high affinity binding to heparin, measured using heparin plates and Micro-cantilever biosensors. Moreover, the peptide bound to HS-rich CHO-K1 cells but not to the HS-deprived CHO-pgd745 cells. The study of the synthetic peptides of the other two putative HBDs is in progress. Heparins probably act also by competing with endogenous HS to bind the BMP6 receptors, suggesting that hepatic HS are involved in hepcidin expression. This is supported by preliminary data showing that the treatment of hepatic cells with inhibitors of HS biosynthesis, such as sodium chlorate and siRNAs for Ext1, Ext2 and Ndst1, strongly reduced hepcidin and BMP6/SMAD signaling. Preliminary data in vivo showed that liver-specific suppression of Ndst1 in mice also strongly reduced hepcidin expression, but the effects on iron homeostasis has not been studied yet. This has been analyzed in transgenic mice with heparanase overexpression, which showed higher levels of serum and liver iron caused by the reduced levels of hepcidin. In summary our preliminary data seem to support a role of liver HS in hepcidin regulation and further studies are needed.

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CRITICAL ROLE OF GALECTIN-3 IN INFLUENZA VIRUS INFECTION BY REGULATING THE HOST IMMUNE RESPONSES

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Galectin-3 is a β-galactoside binding animal lectin widely distributed in immune and epithelial cells. Galectin-3 has been reported to regulate various immune functions and also participate in microbe infection. H5N1 is a highly pathogenic avian influenza virus that causes pneumonia and acute respiratory distress syndrome in humans. Virus-induced excessive host immune responses, such as cytokine dysregulation, contribute to the pathogenesis of human H5N1 disease. In the present study, we investigated the roles of endogenous galectin-3 in H5N1 virus-induced host immune responses using wild-type (WT) and galectin-3 knockout (Gal-3KO) mice. In a mouse model, we observed that galectin-3 was up-regulated in the lung after H5N1 virus infection, and increased galectin-3 were mainly contributed by the infiltrating cells. Our preliminary data also showed that Gal-3KO mice were less susceptible to H5N1 virus infection compared to WT mice, whereas the viral loads in the lung were comparable. On the other hand, we found that H5N1-infected Gal-3KO mice exhibited lower degree of lung inflammation and immune cell infiltration. Moreover, in comparison to WT mice, the level of proinflammatory cytokine IL-1β in both bronchoalveolar larvage fluid and the lung were significantly reduced in Gal-3KO mice during H5N1 virus infection. In the *in vitro* study, we also showed that bone marrow-derived macrophages from Gal-3KO mice produced lower levels of IL-1β and the chemokine MCP-1 compared to those from WT mice in response to H5N1 infection. Collectively, our results suggest that galectin-3 may enhance the pathological effects of H5N1 virus infection by promoting the host inflammatory response and that galectin-3 may serve as a therapeutic target for H5N1 virus-induced severe lung inflammation.

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CHOLERA TOXIN BINDING INFLUENCED BY MIXED GANGLIOSIDES: A BINDING COOPERATIVITY STUDY USING A HIGH-THROUGHPUT CELL MEMBRANE ARRAY

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Lectins often consist of multiple binding subunits that exhibit specific or semi-specific glycan recognition. The cooperative action between multiple bound ligands often strongly enhances the binding avidity and specificity. Although the binding subunits of lectins are often identical and many lectins preferentially bind to the same glycan structure, they still exhibit unique binding patterns to various cell surfaces. We hypothesize that the unique lectin binding patterns on heterogeneous cell surfaces are achieved via cooperative interaction between bound glycan moieties.

Recently, we studied the classic pentameric lectin, cholera toxin subunit B (CTB), binding to GM1-like gangliosides (GM1, fucosyl-GM1, and GM2). This study utilized a nanocube-based sensor with a cell membrane mimicking lipid bilayer. This novel sensor is an ideal tool for studying binding cooperativities because glycans can freely diffuse and rotate on 2D fluidic cellular membranes allowing glycan self-organization to enable multivalent interactions. This label-free sensing platform can be conducted in standard 384-well microplate; therefore, its high-throughput utility enables the complex analysis of multivalent CTB binding.

We performed experimental measurements and theoretical analysis of a stepwise binding model to investigate the influence of cooperativity on CTB binding. Two important phenomena were found. First in contrast to GM1, the cooperative interaction between bound fucosyl-GM1 molecules is negative. Surprisingly, such negative binding cooperativity increases the binding capacity of CTB on a cell membrane surface. We confirmed and explained this result with a computational model of a stepwise binding mechanism. Second, we found the strong binding ligand (fucosyl-GM1) could activate the very weak binding ligand (GM2). A fucosyl-GM1/GM2 mixture increased the maximum binding of CTB on membrane surfaces. As such, the attenuation or enhancement of CTB binding is not simply controlled by the concentration of strong ligands; the cooperative actions among gangliosides in a complex can influence the overall binding. These unexpected discoveries not only demonstrate the essence of cooperativity in the multivalent lectin binding, but also significantly impact lectin-based glycomic analysis. In summary, our nanocube-based cell membrane array provides an excellent tool to dissect complex multivalent interactions; its easy-to-use and high-throughput features will make this tool immediately available to biological communities.

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COMBINING 3D STRUCTURE WITH GLYCAN ARRAY DATA PROVIDES INSIGHT INTO THE ORIGIN OF GLYCAN SPECIFICITY

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Powerful insight into biological recognition mechanisms can be gained from 3D structures of glycan binding proteins (GBPs) complexed to glycans; however such structures remain difficult to obtain experimentally. Here an automated 3D structure generation technique, called computational carbohydrate grafting, is combined with the wealth of specificity information available from glycan array screening. Integration of the experimental array data allows generation of putative co-complex models that can be objectively assessed and iteratively altered until a high level of agreement with experiment is achieved. When applied to a collection of ten GBP-glycan complexes, for which crystallographic and array data have been reported, grafting provided a structural rationalization for the binding specificity of more than 90% of 1,223 arrayed glycans.

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THE GCNT2/GALECTIN-3 AXIS REGULATES MELANOMA MALIGNANCY

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Malignant transformation is often associated with aberrant glycosylation of cell surface glycoproteins and glycolipids. Commonly observed changes in glycan structures during malignancy include increased presence of sialic acid, altered expression and O-linked glycosylation of mucins, and abnormal branching of N-glycans. Increasing evidence supports the hypothesis that the presence of certain glycans correlates with cancer progression by affecting tumor cell invasiveness, binding to pro-tumorigenic galectins, and promoting metastasis to distant organs. Taking this premise into consideration, we conducted MALDI-TOF mass spectrometry on cell surface glycans from normal human epidermal melanocytes (NHEM) and metastatic melanoma (MM) cells as well as comparative glycomic gene profiling of NHEM and MM cells. We found that a defining feature between NHEMs and MMs was the relative level of "I"-branched and "i"-linear poly-N-acetyllactosamines on N-glycans. While NHEM predominantly expressed "I"-branched structures, MMs variably expressed both "i"-linear and "I"-branched modified N-glycans. Furthermore, glycomic gene profiling and confirmatory RT-qPCR data showed that the "I"-branching ß1,6 N-acetylglucosaminyltransferase 2, GCNT2, is consistently increased in NHEM compared with MMs. With regards to its role in malignancy, we found that GCNT2 functioned as a negative regulator of galectin-3 binding. GCNT2 overexpression in human MM cells inhibited galectin-3 mediated adhesion, while GCNT2 knockdown in human MM cells promoted galectin-3 mediated adhesion. In addition, *in vivo* growth of GCNT2-overexpressing and GCNT2-knockdown human MM cells in immunodeficient NOD-Scid/IL-2Rγ-/- mice was significantly altered. These findings highlight new glycobiological insights into melanoma development, implicating GCNT2 as a negative regulator of galectin-3 binding and melanoma malignancy.

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SYNTHESIS OF LACDINAC COMPOUNDS USING A BACTERIAL GLYCOSYLTRANSFERASE

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The LacdiNAc (GalNAcβ-1,4-GlcNAc) epitope has a low abundancy in human cells but some studies have shown cancer-dependent up- or down- regulation of its expression. The LacdiNAc epitope is also overexpressed in helminth parasites. Galectin-3 was reported to bind to LacdiNAc and mediate immune recognition. Additionally, it was shown that the LacdiNAc epitope is recognized by an adhesin from *Helicobacter pylori* which may play a role in bacterial colonization in the gastric mucosa. Synthetic LacdiNAc derivatives would be useful to study their interactions with lectins such as the *Wisteria floribunda* agglutinin. We have used a bacterial β-1,4-galactosyltransferase from *H. pylori* (HP0826) to synthesize *p*-nitrophenyl LacdiNAc from *p*-nitrophenyl *N*-acetyl-glucosaminide. A large excess (5-10X) of donor was necessary to drive the reaction to completion since the natural donor for this galactosyltransferase is UDP-Gal. The preparative reaction was coupled with the UDP-GlcNAc/Glc 4-epimerase (Gne or Cj1131c) from *Campylobacter jejuni* in order to be able to use UDP-GlcNAc as a less expensive precursor of the donor, UDP-GalNAc. In this way, we synthesized 20 mg of *p*-nitrophenyl LacdiNAc with a recovery yield >85%. The product was characterized by thin layer chromatography, capillary electrophoresis (PDA detector), mass spectrometry and NMR.

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IN VIVO TAGGING OF NON-CELLULOSIC PLANT CELL WALL GLYCANS USING TAGGED CBM AND SCFV PROBES

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We have generated a large and diverse collection of monoclonal antibodies (MAbs) that bind to diverse structures within the non-cellulosic glycans of the plant cell wall. These MAbs have been widely used to localize plant cell wall polysaccharides in fixed tissues and cells. It would be advantageous to be able to localize cell wall polysaccharides in living tissues in order to better study cell wall dynamics in growing and developing plants. We have cloned the variable domains of the heavy and light immunoglobin chains of plant glycan-directed MAbs from hybridoma lines that secrete these antibodies. So far, both variable domains from 94 MAbs have been cloned. Comparisons of the sequences of the cloned variable domains show interesting relationships between the variable domains of many of the heavy and light chains that might be correlated to the glycans recognized by the parent MAbs. The cloned variable domain sequences are being used to create single-chain variable-fragments (scFvs) that include the two immunoglobin variable domains linked by a flexible connector peptide. These scFvs are being expressed in heterologous systems to determine if they retain the abilities of the parent MAbs to recognize their glycan ligands in ELISA and immunohistochemical tests. Genes encoding these scFvs, as well as those encoding carbohydrate binding modules (CBMs), are being fused to fluorescent protein tags for transformation into Arabidopsis in order to determine if their expression leads to labeling of cell walls in the transgenic plants or affect plant growth and development. Results thus far suggest that these probes are able to label plant cell walls *in vivo*, in some cases without causing growth or developmental abnormalities. In other cases, *in planta* expression of these glycan-directed probes results in growth and/or developmental changes in the transgenic plants. The long-term goal of these studies is to provide cloned antibody sequences that can be used either to monitor the dynamics of cell wall polysaccharides in living plant cells or to selectively target cell wall polysaccharides to disrupt their function in the plant cell wall. *[Supported by a grant from the NSF Plant Genome Program (IOS-0923992)].*

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POLAR ENRICHMENT OF GLYCOSYLTRANSFERASES INVOLVED IN THE CELL ENVELOPE BIOGENESIS IN MYCOBACTERIUM SMEGMATIS

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Mycobacteria have a thick, multilaminar cell envelope, which is critical for viability and pathogenesis during mycobacterial diseases such as tuberculosis. The cell envelope is composed of glycolipid-rich outer membrane linked to the arabinogalactan-peptidoglycan core, and an innermost plasma membrane containing unique phosphatidylinositol (PI)-anchored mannans. While many pathways of cell envelope glycan biosynthesis have been clarified, the regulation and coordination of these pathways with cell growth remains largely unknown. Furthermore, recent studies suggest that mycobacteria grow from their polar ends, implying that the synthesis of new cell envelope glycans requires spatiotemporal regulation. We previously isolated two distinct membrane fractions from *Mycobacterium smegmatis* by density gradient fractionation: 1) the PM-CW, plasma membrane tightly associated with the cell wall, and 2) the PM_f, a pure membrane free of the cell wall components. Furthermore, we showed that the early and late steps of PI-anchored mannan biosynthesis are compartmentalized into the PM_f and PM-CW membrane fractions, respectively. Nevertheless, our knowledge of this mycobacterial membrane compartmentalization remains limited. In this study, we first used comparative proteomics to reveal a comprehensive list of proteins associated with the PM_f or the PM-CW. Among the list of the PM_f–associated proteins, we experimentally verified the specific PM_f binding of various glycosyltransferases, such as PimB', a mannosyltransferase involved in the biosynthesis of PI-anchored mannans; Ppm1, a subunit of polyprenol-phosphate-mannose synthetase; and GlfT2, a galactosyltransferase involved in arabinogalactan biosynthesis. Live-imaging fluorescence microscopy further showed that the PM_f is associated with the nascent pole of the growing cells *in vivo*, while the PM-CW appeared to be the canonical plasma membrane. Thus, our data suggest that the PM_f is a metabolically active, multifunctional membrane associated with polar elongation of mycobacterial cell. Comprehensive lipidomic analysis of these membranes is currently underway.

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GLYCAN LIGANDS FOR TARGETING SIGLECS ON IMMUNE CELLS

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Sialic acid binding immunoglobulin lectins (Siglecs) are a family of cell surface receptors expressed on one or a few immune cell types and as such are well suited as targets for cell directed therapies. A challenge for targeted delivery is to identify ligands of high selectivity and avidity to bind to a single member of the siglec family, which now comprises 15 members in man and 9 in mouse. Siglecs bind to sialic acid containing glycans and it has been shown that substituents at C-9 of Neu5Ac can increase the selectivity and affinity of ligand binding.

To identify ligands we have chemo-enzymatically synthesized a diverse library of analogs utilizing highly efficient sulfonamide chemistry to introduce substituents to the C-9 position of sialic acid linked either alpha2-3 or alpha2-6 to LacNAc-ethyl azide. Using a microarray format, the glycan analog library was screened against a panel of siglec-Fc's to identify ligands of high avidity and selectivity. Several lead compounds were identified from the library and conjugated to lipids for display on liposomal nanoparticles to evaluate targeted delivery to cells. Liposomes decorated with a sulfonamide ligand for Siglec-F were found to target fluorescent liposomes to Siglec-F expressing cells. Using these novel sulfonamide analogs we have further demonstrated the utility of siglec ligand decorated nanoparticles for targeting siglec expressing cells. (NHLBI grant HL107151).

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GD3 IDENTIFIES GLIOBLASTOMA STEM CELLS AND GD3 SYNTHASE IS A KEY DRIVER FOR TUMORIGENICITY

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Cancer stem cell (CSC) is a small population of cancer cells possessing self-renewal ability and tumor initiation. Accumulating evidence shows that the cancer stem cells of glioblastoma multiforme (GBM), a grade IV astrocytoma, can be enriched by the expressed marker CD133, and CD133-positive cells are capable of self-renewal and recapitulation of the brain tumor when transplanted into immunodeficient mouse brain. However, recent studies have shown that CD133 is not exclusively expressed on GBM stem cells (GSCs) as CD133-negative cells also possess tumor-initiating potential. Here we show that ganglioside GD3 can be used as additional marker to further enrich the GBM cancer stem cell population with remarkable capability of self-renewal and tumorigenicity. We found that GD3 was significantly overexpressed on eight neurospheres and tumor cells, and in combination with $CD133^{hi}$, the sorted cells exhibited a higher expression of stemness genes and self-renewal potential in vitro, as well as tumor initiation in vivo. Furthermore, GD3 synthase (GD3S), an alpha-2,8-sialyltransferase involved in the synthesis of GD3 from GM3, is increased in neurospheres and in human GBM tissues, but not in normal brain tissues. Suppression of GD3S resulted in decreased GSC-associated properties, and GD3 antibody was shown to induce complement-dependent cytotoxicity against cells expressing GD3. Our results demonstrate that GD3, regulated by GD3S, is highly expressed in the GSC population, and that GD3 and GD3S are potential therapeutic targets against GSC and GBM.

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GALECTIN-3 BINDING PROTEIN AND GALECTIN-1 INTERACTION IN BREAST CANCER CELL AGGREGATION AND METASTASIS

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Galectin-3 binding protein (Gal-3BP) is a large hyperglycosylated protein that acts as a ligand for several galectins through glycan-dependent interactions. Gal-3BP can induce galectin-mediated tumor cell aggregation to increase the survival of cancer cells in the bloodstream during the metastatic process. However, the galectin interacting with Gal-3BP and its binding specificity has not been identified and structurally elucidated, mainly due to the limitation of mass spectrometry in glycan sequencing. To understand the role of Gal-3BP, we here used liquid chromatography-mass spectrometry combined with specific exoglycosidase reactions to determine the sequences of *N*-glycans on Gal-3BP from MCF-7 and MDA-MB-231 cells, especially the sequences with terminal sialylation and fucosylation, and addition of LacNAc repeat structures. The *N*-glycans from both strains are complex type with terminalα2,3-sialidic acid and core fucose linkages, with additional α1,2- andα1,3 fucose linkages found in MCF-7 cells. Compared with that from MCF-7, the Gal-3BP from MDA-MB-231 cells had fewer tetra-antennary structures, onlyα1,6-linked core fucoses, and more LacNAc repeat structures; the MDA-MB-231 cells had no surface galectin-3 but used surface galectin-1 for interaction with Gal-3BP to form large oligomers and cell aggregates. This study elucidates the specificity of Gal-3BP interacting with galectin-1 and the role of Gal-3BP in cancer cell aggregation and metastasis.

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USING RESET AND SIALOSYL IODIDE GLYCOSYLATION TO DEVELOP CHEMICAL PROBES TO STUDY SIALIC ACID (NEU5AC) BIOCHEMISTRY

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In mammalian cells, glycans comprised of sialic acids play an important role in various biochemical pathways. Sialic acid is a family of more than 50 forms and *N*-acetyl neuraminic acid (Neu5Ac) is the most common form in mammalian cells. In recent years, reports show that partially *O*-acetylated Neu5Ac derivatives pose diverse functions in bacteria and mammalian cells. However, harsh extraction conditions and labile/migration properties of the *O*-ester groups limit accessible quantities for biochemical studies where purity is a virtue. To overcome this challenge, a series of partially *O*-acetylated Neu5Ac were synthesized using regioselective silyl exchange technology (ReSET) to serve as standards and substrates for chemoenzymatic studies that can lead to unique sialoglycoconjugates. In addition, a novel sialosyl iodide was developed to provide a chemical methodology by which α-sialoglycoconjugates andβ-sialosterolscould be prepared in pure forms. Sialosyl iodide serves as a donor that gives exclusively α-linked andβ-linkedsialosides. α andβ-Sialosterols have been shown to have neuritogenic activity in NIE.115 neuroblastoma cells. In conjunction with the developed methodologies, understanding the role of sialic acid in biological systems can be explored further.

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FUNCTIONAL LANDSCAPING OF THE CELL SURFACE GLYCOME

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Cell surface glycans lie at the interface between the cell and its environment, playing an active role in mediating interactions with external substances. A better understanding of their functional roles is achieved by structure-specific re-engineering towards specified glycosylation patterns. Changes in the glycan landscape (glycoscaping) were induced on live cell models, using a mixture of glycosidases, glycosidase inhibitors, and glycosyltransferase inhibitors. Following treatment, an in-depth glycomic analysis was performed to characterize the exact changes that occurred. With this knowledge, cells were evaluated to determine how glycan changes affect their functions and properties. Treatment of intestinal epithelial cells showed differences in membrane enzyme activity, cell permeability, and microbial adhesion and infection compared with untreated controls.

Although current methods enable detection of specific glycan epitopes, detailed structural information is needed to better assess their involvement during interactions with other molecules. Mass spectrometry-based glycomics has the potential to provide a single platform to monitor hundreds of cell membrane glycans simultaneously and comprehensively. To enable characterization of cell surface glycans, we applied mass spectrometric profiling methods to extracted cell membrane compartments of intestinal epithelial cells. In the highly glycosylated environment of the gut, glycosylation patterns appear to serve as key indicators of the cell state in several ways: (1) During the process of differentiation, a transition from high mannose type to sialylated complex type glycans is observed as the most abundant glycans presented on the cell surface. (2) Glycans on the surface of intestinal cells are available as substrates for bacterial consumption during infection. (3) Glycosylation is sensitive to its micro-environment, where specific analytes added to the media resulted in significant alterations in the glycan profile. (4) In older cells with higher passage numbers, a redistribution of glycans across the cell surface was readily observed. The ability to produce specifically glycoscaped surfaces will provide the means to probe the participation of glycans during cellular processes and disease states. To this end, glycomic analysis has been employed to define key structures.

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CELL SURFACE N-GLYCANS REMODELED IN VITRO USING COMMERCIAL GLYCOSIDASES

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Glycan-protein interactions are involved in different biological processes including development, immunity, inflammation and cancer. As a result, manipulation of cell surface glycans is an essential tool for understanding the mechanisms by which glycans govern cell fate. Traditional approaches using gene KOs, gene silencing, or metabolic blockers are not always adequate, as pleiotropic effects are common when metabolic inhibitors block glycosylation pathways. Targeted approaches like site-directed mutagenesis or gene silencing are not available for every cell type or organism, limiting research on this area.

Specific glycosidases can effectively remove selected glycan residues and/or epitopes at the cell surface; these enzymes could be a viable alternative to remodel the cell surface glycome when other methods are not suitable. Although this approach has been reported in the literature, there has never been a systematic study (comparing different cell lines, enzymes, and reaction conditions) where the extent and efficacy of glycan removal was evaluated along with a measurement of cell integrity and viability. Here, we present optimized conditions (buffer-media-enzyme combinations) for an efficient in vitro cell surface glycan remodeling in different cell types, while monitoring culture viability. After enzymatic treatment, modifications in the glycan structure were analyzed by flow cytometry using specific lectins.

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IDENTIFYING THE MOLECULAR DETERMINANTS OF N-LINKED GLYCOSYLATION IN CAMPYLOBACTER JEJUNI

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N-linked glycosylation is a posttranslational modification system found in eukaryotes and prokaryotes. This modification is found on a diverse, but specific, set of proteins and can influence a variety of biological processes including cell signaling, protein complex assembly and adherence. The specificity of this process is regulated by several factors, including cellular localization, a consensus sequence on acceptor proteins, and solvent accessibility of the modification site. Eukaryotic N-linked glycosylation is a tightly regulated process that modifies proteins that enter the endoplasmic reticulum (ER) lumen through the general secretory pathway (Sec) and that contain a glycosylation consensus sequence (N-X-S/T). Prior to folding, acceptor proteins are modified either co-translocationally, as they pass through the ER membrane, or post-translocationally, prior to folding. Less is known about the spatial and temporal aspects of the more recently discovered N-linked glycosylation pathway of bacteria. Campylobacter jejuni, a Gram-negative foodborne pathogen, harbors a well-studied N-linked glycosylation system that has been implicated in intestinal colonization of the host. Although in vitro experiments with the C. jejuni oligosaccharyltransferase, PglB, demonstrate a preference for acceptor proteins with consensus sequences (D/E-X1-N-X2-S/T, where X is any amino acid except Proline) in flexible, solvent exposed, regions, several native glycoproteins are known to harbor consensus sequences within structured regions of the acceptor protein, suggesting that unfolding or partial unfolding may be important for efficient glycosylation in vivo. To address this, we assessed the glycosylation efficiencies of folded and unfolded acceptor proteins in vitro and in vivo. Through this work, we found that destabilization, or partial unfolding, of acceptor proteins increases glycosylation efficiencies in vitro. Moreover, preliminary studies indicate that fully folded substrate proteins are not efficiently glycosylated in vivo, and suggest that, similar to the eukaryotic system, coupling between the bacterial Sec translocon and PglB may occur. These studies have begun to extend our understanding of the molecular mechanisms underlying N-linked glycosylation in bacteria.

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UNRAVELING THE COMPLEXITY OF GLYCOSYLATION THROUGH SYSTEMS BIOLOGY

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Glycosylation is essential for many biological processes, and diverse glycans that modulate these processes are synthesized through the concerted effort of hundreds of enzymes. Because of the complexity of the glycan biosynthetic pathways, it has been difficult to fully understand the molecular basis of variations in the glycome. To address this challenge, we have developed large computational models of the biomolecular processes underlying glycan synthesis, including glycosylation, metabolism, and the secretory machinery. These models allow us to account for environmental and genetic bases of glycosylation. We have applied these models to aid in cell line characterization and glycoengineering in Chinese hamster ovary (CHO) cells. Specifically, have predicted and experimentally validated the impact of gene knockouts on N-linked glycosylation. Through these validations, we have demonstrated that our approach accurately predicts glycoprofiles across different cell lines (CHO-GS, CHO-S), secreted proteins (EPO, IgG, and the endogenous secretome), glycoprofiling techniques (HPLC, MALDI-MS), and genome editing techniques (Zinc-finger nucleases and CRISPR-Cas). Furthermore, by augmenting our glycosylation modeling framework with a model of metabolism including all known metabolic pathways, we can analyze the effects of additional factors on glycan synthesis in CHO cells, including variations in media composition, mutations in different cell lines, and potential gene deletion mutants. Lastly, we have leveraged this platform to study subcellular glycosyltransferase localization. Thus, this modeling platform can be invaluable for guiding engineering efforts in cell factories for biopharmaceutical and biosimilar development, and provide testable hypotheses in this complex, but essential biological system.

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PGLYCOFILTER: A TOOL TO AID IDENTIFICATION OF PLANT N-LINKED GLYCANS FROM MASS SPECTROMETRY DATA

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Proteomics has helped to solve unanswered questions in plant biology. *N*-glycoproteomics holds promise to further increase our understanding of processes related to the plant secretory system and its endpoints; however, analytical challenges have stunted growth in this area. Mass spectrometry is the preeminent technology for analysis of protein *N*-linked glycosylation. Data analysis is necessarily carried out with both protein and glycan databases. These databases can be acquired from a number of online sources. Protein databases are available for many organisms across the kingdoms of life; however, there is no comprehensive database for plant *N*-linked glycans. A thorough review of plant *N*-linked glycan biosynthesis was conducted and revealed that plant *N*-linked glycans contain hexose (0-9), *N*-acetylglucosamine (GlcNAc) (1-4), fucose (0-3), and xylose (0-1). Glycosyltransferases and glycosidases have strict substrate specificities, limiting the possible number of *N*-glycan structures. For example, *N*-acetylglucosaminyltransferase I acts specifically on $GlcNAc_2Mannose_5$ and a terminal GlcNAc is required for the activity of fucosyl- and xylosyltransferase. The results were assembled into a set of rules by which putative *N*-linked glycans, predicted from experimental data with GlycoMod, could be assessed. We automated this process with a tool called pGlycoFilter, implemented in R. The accuracy of pGlycoFilter was tested against a set of complex *N*-linked glycans reported in the literature for which the structures were determined experimentally. Each of these glycans passed pGlycoFilter. We used pGlycoFilter to facilitate interpretation of our experimental data of a previously unstudied peroxidase from palm tree leaves. Of the 290 glycan compositions predicted by GlycoMod for the palm peroxidase, 81 of them passed pGlycoFilter. This list of glycans could be used in a subsequent glycopeptide identification experiment. The described tool, pGlycoFilter will aid in plant glycoproteomics studies by decreasing time need for manual validation of predicted glycan compositions, and will reduce the chance of human error associated with manual interpretation. The growing realization that *N*-glycosylation in plants deserves more attention will necessitate that new analytical platforms for studying plant glycoproteins, such as that developed for this study, are needed in the field of plant biology.

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LINKAGE-SPECIFIC SIALIC ACID DERIVATIZATION FOR MALDI-TOF-MS PROFILING OF IGG GLYCOPEPTIDES

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Glycosylation is the most complex and arguably among the most influential post-translational modifications on immunoglobulin Gs (IgGs). Not only the glycosylation site occupancy is important for the properties of a glycoprotein, but also the type of monosaccharides comprising the glycan and their linkages. For example on IVIG, alpha2-6-linkage of an *N*-acetylneuraminic acid to a neighboring galactose is associated with anti-inflammatory activity, whereas alpha2-3-linkages on recombinant IgGs do not show this effect [1]. Furthermore, a decrease in galactosylation of IgG glycans is linked to an increase in disease activity [2]. Glycosylation is also an important determinant of efficacy and clearance of biopharmaceuticals such as IgG, making careful analysis and control of glycosylation during the production a prerequisite to ensure proper activity [3].

Mass spectrometric analysis of glycopeptides enables us to study glycosylation in a protein- and site-specific way. Matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) is a fast and convenient method for glycopeptide analysis. Using this method, however, information on monosaccharide linkages is often difficult to obtain and the analysis of sialylated glycoconjugates is biased by metastable decay [4]. We present here the specific derivatization of the carboxylic acids on both the peptide and the glycan moieties of glycopeptides, employing the carboxylic acid activator 1-ethyl-3-(3-dimethylamino)propyl)carbodiimide (EDC) and catalyst 1-hydroxybenzotriazole (HOBt) as reported before for released glycans [5]. In contrast to ethanol used for released glycans, dimethylamine showed more promise as a nucleophile for IgG glycopeptides. The reaction was optimized for IgG glycopeptides to perform in a selective way for differently linked sialic acids, introducing a mass difference between alpha2-3- and alpha2-6-linked species. In addition, reactions were controlled to perform fully selective derivatization of the carboxylic acids in the peptide moiety.
The presented method is fast, has an excellent intra- and inter-day repeatability and makes use of relatively inexpensive chemicals. We envision the use of this method for future site-, subclass- and sialic acid-linkage specific glycosylation profiling of therapeutic antibodies, as well as for biomarker discovery in clinical IgG samples derived from plasma.

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LECTIN MICROARRAY ANALYSIS REVEALS IMPACT OF MICROBICIDE FORMULATION ON GLYCOSYLATION AND INNATE IMMUNITY IN CERVICOVAGINAL FLUID

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Glycosylation plays a crucial role in the establishment of healthy symbiotic bacterial colonization patterns, the maintenance of mucosal barriers to infection, and regulation of both adaptive and innate immunity. Changes in glycosylation state have powerful effects on antibody function and protein stabilization and can alter microbicidal effects of immune molecules. Thus, the glycome is both a modulator and marker of innate immunity. In recent work, we have used lectin microarray analysis to demonstrate a strong correlation between glycosylation status in cervical vaginal lavage (CVL) fluids with microbiome status (PLOS One, **2015,** *10*, e0127091). Here we report on the impact of thin film versus hydrogel placebos for vaginally-delivered drugs (e.g. microbicides) on the glycosylation and innate antiviral immunity of CVL fluids. We studied CVL samples from 53 women using lectin microarray analysis and studied their impact on the infectivity of herpes simplex viruses 1 & 2 (collectively HSV) and HIV-1. In our studies, lower levels of high mannose, as indicated by the high mannose binding lectins griffithsin (GRFT) and scytovirin (SVN), were correlated with both lower anti-HIV-1 and anti-HSV-1/2 activities ($p<0.01$ and $p<0.001$ respectively, n= 90 samples, t-test). Our studies also found a significant impact of drug delivery mode on glycan composition in the CVL that in turn may impact innate immunity. Gel use had a greater impact on the glycome, with a loss of high mannose predominant in the signature ($p<0.05$, n=53 patients, paired t-test) We observed a similar loss of high mannose (reflected in GRFT and SVN binding) with the use of thin films, although the impact was lower and not statistically significant ($p\sim0.4$). Pulldown of GRFT-binding glycoproteins in the CVL identified a number of antiproteases that are associated with reduced susceptibility to HIV. Taken together our data suggests that high mannose is a signature of anti-viral actvitiy in the CVL and that application of gels vaginally may negatively impact innate antiviral immunity.

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A MIRNA PROXY APPROACH TO DECODING THE GLYCOME

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Glycosylation has a vast capacity for encoding biological information and informing biological function. Our ability to decode the functional components of glycosylation in specific biological systems is attenuated by the difficulty in pinpointing specific glycans and corresponding biosynthetic enzymes involved. MicroRNAs (miRs) are small non-coding regulatory RNAs that bind cognate mRNA, inhibiting either stability or translation of the transcript. MiRs regulate networks of genes that work in concert to control a specific biological process, tightening the expression window for critical genes. In previous work, our laboratory identified miRs as major regulators of glycosylation (*PNAS,* **2014,** *111,* 4338-43*)*. Several studies have found that inhibition of miR-targeted glycogenes map onto biological effects associated with the miR, suggesting that miRs identify critical glycogenes within a biological pathway. Herein we present data from two miR systems that demonstrate the ability of miRs to identify the involvement of specific glycans in biological processes; miR-200f, a regulator of epithelial to mesenchymal transition (*PNAS,* **2015**, *112*, 7327-32) and miR-424, a regulator of cell cycle in breast epithelia. This is a powerful concept as it is difficult to pinpoint which glycogenes are important in disease due to both the complex biosynthetic pathways of the glycome and the fact that changes in glycan structures often do not map directly onto changes in mRNA, analysis of which is further complicated by low transcript abundance. Analysis of miR networks is a relatively simple proxy to decrypt which glycogenes, including those encoding difficult to analyze structures (e.g. proteoglycans, glycolipids), are responsible for specific changes important in disease.

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UNUSUAL STRUCTURAL FEATURES OF EXTRACELLULAR POLYSACCHARIDES AND GLYCOPROTEINS IN THE HYDROCARBON-PRODUCING ALGA BOTRYOCOCCUS BRAUNII

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Botryococcus braunii is a colonial green alga, which has potential as a bioenergy source since it produces liquid hydrocarbons that can be readily converted into conventional combustion-engine fuels. The cells of the algal colony associate through a complex extracellular matrix (ECM) and the hydrocarbons are stored in this ECM. The outer edge of the ECM consists of long polysaccharide fibers that are based in a retaining wall, which prevents the liquid hydrocarbons form escaping the ECM. The colonies shed pieces of the polysaccharide fibers and retaining wall, termed "shells", into the media during cell division. As part of our efforts to maximize hydrocarbon production in this alga, there is interest in disrupting the synthesis of the retaining wall and polysaccharide fibers, so that effects of mutations on hydrocarbon production can be evaluated directly and that hydrocarbon extraction can be facilitated. Toward this goal, we carried out structural analysis of the prepared shell material. Our glycosyl composition and linkage analysis on isolated *B. braunii* shells found that the shells consist of 97.9 % carbohydrate, with arabinose (42 %) and galactose (39 %) as the main components. Arabinose was present as terminal and 2-linked furanose, while galactose occurs mostly as 2,3- and 2,4-linked pyranose. Interestingly, we have also detected unusual monosaccharide residues, including 6-deoxyaltrose, 3-methyl-arabinose and 6-methyl-galactose. In addition to the polysaccharide fraction, the shells also contain a 162-kD glycoprotein. We have investigated the oligosaccharide modifications on this 162-kD protein. Enzymatic N-glycan release in the presence of O-18 water, followed by LC-MS/MS analysis indicated that 10 of the 14 glycosylation motifs (-N-X-S/T-) are glycosylated. MS analyses and glycosyl linkage analysis of released N-linked glycans showed that the protein possesses pauci- and high-mannose type N-linked glycans, some of which are fucosylated. Notably, the protein carries a unique N-glycan with fucose and arabinose. Overall, our comprehensive analyses of *B. braunii* shells contributed to a better understanding of the structural details of the shell content, which is essential for utilizing the species as a bioenergy source.

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HIGH THROUGHPUT ANTIBODY ANALYSIS USING RAPID DEGLYCOSYLATION, DIRECT LABELING, AND PROTEASES

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Monoclonal antibody characterization requires the complete release of glycans from conserved and non-conserved sites, a step that often takes several hours and could compromise the integrity of the polypeptide chain. To optimize this workflow, we have developed Rapid PNGase F for complete and unbiased release of N-glycans in only five minutes, which was also used in combination with other exo-glycosidases, endo-glycosidases, and/or O-glycosidase. These protocols were developed to release all N- and O-glycan chains from heavily glycosylated molecules (i.e. etanercept), or to analyze site occupancy with endoglycosidases (EndoS, EndoD).

These methods were optimized to achieve complete deglycosylation while preserving the integrity of the antibody's tetrameric structure, enabling downstream applications using intact protein mass analysis. In combination with high purity proteases, these methods streamline standard procedures for antibody characterization.

Finally, we present methods where glycans are efficiently labeled (with a tag of choice) in a reaction immediately following enzyme digestion, avoiding biases caused by glycan purification steps. This innovative technique does not require an intact glycosylamine at the reducing end, therefore it is compatible with enzymes working at low pH (such as PNGase A).

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GLYCOPEPTIDE ANALOGUE OF PSGL-1 BINDS TO P-SELECTIN WITH NANOMOLAR AFFINITY IN VITRO

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Selectins, as the mediator of early leukocyte trafficking and intracellular signaling in the inflammatory cascade, have been a popular target for the design of therapeutic agents that alter the progression of inflammatory responses. Antibodies, small molecular inhibitors and glycomimetics that block the interaction of P-selectin and P-sel-glycoprotein-ligand-1 (PSGL-1) have been developed but are limited by low potency or high cost. Previously, glycosulfopeptides (GSP-6) that mimicked the tyrosine sulfate- and sLex-containing terminus of PSGL-1, the recognition site of selectins, were shown to bind to P-selectin with relatively high affinity. However, the production of GSP-6 was difficult due to the acid sensitivity of tyrosine sulfates. Here, replacing the sulfate (SO3-) with sulfonate mimics (CH2SO3H or SO3H), we chemically synthesized core-2 glycosulfonatepeptides and then incorporated the sLex moiety by sequential enzymatic reactions. To evaluate the binding of glycosulfonatepeptides (GSnPs) with selectins, a microarray containing the intermediates and final products (named GSnP-6 and GSn2P-6) were generated. GSPs were included in the microarray as the native comparison. We first verified the success of the synthesis by the binding of various lectins and antibodies specific for the glycan moiety and peptide backbone. Next, recombinant chimeras of human and mouse P-, L- and E-selectin were screened on the glycopeptide microarray. The GSnP-6s bound to all selectins similar to the GSP-6, indicating the substitution of tyrosine sulfate did not interfere the selectin recognition. The binding kinetic constants of GS(n)P-6s to recombinant P-selectins were then determined by surface plasma resonance. The three GS(n)P-6s all bound to human P-selectin with nanomolar affinity (Kd=9-22 nM), while the Kd for the mouse P-selectin was approximate one magnitude higher. Consistent with literature reports, GS(n)P-6s were able to bind to E- and L-selectins but at a much lower affinity. Our in vitro data suggested that GSnP-6 could potently inhibit the interaction of P-selectin with PSGL-1, which was later confirmed by a series of in vivo studies. Therefore, GSnP-6 represents a promising candidate for the treatment of diseases driven by acute and chronic inflammation.

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ISOTOPE TARGETED GLYCOPROTEOMICS FOR INTACT N- AND O-GLYCOPEPTIDE DISCOVERY

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Glycosylation of proteins generates greater proteomic diversity than any other post-translational modification (PTM) and plays an essential role in biological regulation. However, the diversity found in glycoproteins results in limitations to detection and identification that have thus far undermined efforts to describe the intact glycoproteome via mass spectrometry (MS). Isotope Targeted Glycoproteomics (IsoTaG) is a mass-independent chemical glycoproteomics platform for characterization of intact, metabolically labeled glycopeptides at the whole proteome scale. IsoTaG utilizes isotopic recoding to generate a glycan-specific perturbation in them/z spectrum. The unique perturbation is then used as a handle to direct tandem MS and targeted database searching for assignment of glycan structure and peptide sequence from intact N- and O-glycopeptides. The IsoTaG platform comprises (1) metabolic labeling of the glycoproteome with an azidosugar, (2) chemical enrichment and isotopic recoding of glycopeptides using a cleavable probe, (3) directed mass spectrometry, and (4) targeted assignment of intact glycopeptides. The glycan-specific isotopically recoded mass envelopes enable immediate confirmation of enriched glycopeptides by full scan MS. A computational pattern searching program is used to generate an inclusion list for targeted tandem MS that increases selection and high-confidence assignment of glycopeptides. As the IsoTaG approach is readily adapted to any PTM that can be chemically labeled, the platform may be broadly useful for the discovery of low abundance novel protein isoforms, including non-templated PTMs and peptide sequence polymorphisms (pSPs).

IsoTaG has enabled the survey of over ten glycoproteomes from immortalized cell lines, resulting in the identification of hundreds of N- and O-glycopeptides, which represent 16 N- and 11 O-glycan structures. Furthermore, IsoTaG analysis of the O-GlcNAc glycoproteome in resting and activated primary human T cells has revealed hundreds of O-GlcNAcylated peptides from limited sample quantities (less than 0.5 mg protein), many of which represent novel glycoproteins and glycosites. Additionally, we identified unexpected peptide sequence polymorphisms (pSPs) that were not present in the predicted human protein database. Our findings support the emerging notion that cancer cells are replete with glycomic and proteomic signatures that are not encompassed by current databases.

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A MUTANT O-GLCNACASE AS A PROBE TO REVEAL GLOBAL DYNAMICS OF THE O-GLCNAC PROTEOME DURING DROSOPHILA EMBRYONIC DEVELOPMENT

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O-GlcNAcylation is a reversible type of Ser/Thr glycosylation on nucleocytoplasmic proteins, which occurs in all metazoans examined to date. Its cycling on substrates is carried out by two single essential enzymes; O-GlcNAc transferase (OGT), which installs the modification and O-GlcNAc hydrolase (O-GlcNAcase or OGA), which removes it. Various genetic approaches in several animal models have revealed that protein O-GlcNAcylation is required for embryogenesis. Drosophila melanogaster OGT is a polycomb gene, null mutants of which display homeotic transformations and die at the pharate adult stage. The status of global O-GlcNAcylation levels, the identities of the modified proteins involved and the underlying mechanistic biology linking these to embryonic development are poorly understood. One of the limiting factors towards characterizing changes in O-GlcNAcylation has been the limited specificity of currently available tools to detect this modification. In this study, harnessing the unusual properties of a bacterial O-GlcNAcase mutant that binds O-GlcNAc sites with nanomolar affinity, we uncover changes in patterns of protein O-GlcNAcylation in Drosophila embryos (growing at 25 °C) at the cellular blastoderm (2.5 h), germ band extension (5 h), germ band retraction (10 h) and dorsal closure (15 h) stages. We then use the mutant OGA probe as a bait to enrich for O-GlcNAcylated proteins from samples of embryos at various developmental stages (0 – 16 h) and use mass spectrometry to discover novel conserved O-GlcNAcylated proteins, the lack of O-GlcNAcylation of which may contribute to the homeotic phenotypes observed in OGT null Drosophila mutants.

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PHYSIOLOGICAL CONSEQUENCES OF LOSS OF OGT DEPENDENT O-GLCNACYLATION IN HEPATOCYTES

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O-linked-β-N-acetylglucosamine transferase (OGT) post-translationally modifies serine and threonine residues of proteins and is required for stem cell viability and embryogenesis. OGT knockout is early embryonic lethal but not much is known about the different tissue-specific functions of OGT and O-GlcNAcylation in differentiated adult tissues. We have focused on *O*-GlcNAcylation in hepatocyte function in conditional liver-specific OGT knockout mice.

Albumin-Cre driven Ogt knockout mice develop normally. Remarkably hepatocytes are quite resistant to the loss of OGT enabling a model system for proteome-wide analysis of reduced *O*-GlycNAcylation. *O*-GlcNAcylation is drastically reduced in mutant liver tissue in vivo during postnatal development analyzed by WGA chromatography. The loss of OGT in hepatocytes increased cell proliferation compared to control littermates. Co-expressed Cre dependent lineage marker indicates unexpected prolonged survivial of hepatocytes upon loss of OGT activity. In addition we have characterized phosphorylation and acetylation changes in the hepatic proteome in response to loss of OGT activity and effects on metabolic pathways.

Liver Glycogen Synthase (GS) phosphorylation in Ogt deficient hepatocytes is increased at serines 641 and 645 which regulate GS activity in response to blood glucose levels. These data indicate OGT mediated control of glucose immobilization by GS in liver.

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DISRUPTION OF BRAIN O-GLCNAC CYCLING INDUCES OTX2-DEPENDENT DEVELOPMENTAL DELAY

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Due to recent changes in nutritional habits, determining the impact of diet on development is emerging as a critical area for further research. The average American eats around 22 tsp of added sugar per day, thus, understanding what happens when sugars are metabolized will be of utmost importance for public health.

O-GlcNAcylation is one of the key components of diet-responsive signaling. This glucose rheostat is a ubiquitous and dynamic post-translational modification of intracellular proteins. Two key enzymes drive *O*-GlcNAc cycling: the *O*-GlcNAc transferase (OGT), which adds the modification, and the *O*-GlcNAcase (OGA), which removes it. Although many studies have focused on *O*-GlcNAc cycling by modulating the expression or activity of OGT, few studies have targeted hyper-*O*-GlcNAcylation by disturbing OGA. Because this post-translational modification is directly dependent upon glucose input, depleting OGA creates an artificial and constant hyperglycemia-induced *O*-GlcNAcylation state.

Herein, we used *Oga* knockout (KO) cells and mice as a model to decipher the impact of high glucose concentration on development. We generated a brain specific *Oga* KO mouse model using Nestin targeted cre-lox technology. After confirming brain specific OGA depletion, we observed that these mice had small bodies and short faces, microcephaly, increased fat percentage and metabolic abnormalities. Following up on these observations, we correlated this phenotype to a hormonal defect and examined neuroendocrinologic development. We observed that the pituitary gland was smaller in both the embryo and adult mice. Consequently, we hypothesized that pituitary gland maturation was delayed, and failed to support normal hormonal secretion needed for proper development. Therefore, we investigated differentiation of embryonic stem cells into brain lineage. We found that the exit from naïve pluripotency was accelerated for the *Oga* KO stem cells, as compared to wild type stem cells. mRNA expression analysis revealed *Otx2*, a homeobox gene, as a candidate responsible for the deregulation observed in *Oga* KO stem cells. Our experiments have demonstrated that Otx2 mRNA levels are increased and the protein is stabilized following Oga KO in vitro, and expressed longer during brain development throughout embryogenesis. Accordingly, we have demonstrated a developmental delay in the brain-specific *Oga* KO mice, likely due to a deregulation of the temporal secretion of hormones. The hyper-O-GlcNAcylation caused by the *Oga* depletion is likely to affect final differentiation of the pituitary gland, and subsequent hormone secretion. Finally, this study highlights *O*-GlcNAcylation as a novel pathway in which glucose consumption may impact proper embryonic development.

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ANALYSIS OF THE BIOLOGICAL ROLES OF EXTRACELLULAR O-GLCNAC USING EOGT-DEFICIENT MICE

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EOGT is an atypical O-GlcNAc transferase, responsible for extracellular O-GlcNAcylation of epidermal growth factor (EGF)-like domain-containing proteins, such as Notch receptors. Recently, mutations in *EOGT* have been found in patients with Adams-Oliver syndrome, a rare congenital disorder characterized by ischemic changes in brain, vertex scalp defects, and terminal transverse limb defects. Although molecular basis for this syndrome is currently unknown, it could be associated with vascular defects. To clarify the biological function of EOGT and contribution to Notch signaling pathway, we generated a mutant allele for *EOGT*. Unlike reported human phenotype, EOGT mutant mice exhibit no gloss abnormality, suggesting that O-GlcNAc is dispensable for most of the Notch-dependent developmental processes. However, aged *EOGT*–deficient mice exhibits neurodegeneration in the brains. Remarkably, defects in blood-retina/brain barrier were observed in *EOGT*-deficient mice. This phenotype was enhanced under *Notch1*$^{+/-}$ heterozygous background in the retina. Our findings suggest that EOGT may affect Notch signaling in a contex-dependent manner during vascular development.

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O-GLCNAC CYCLING PLAYS A CRUCIAL ROLE IN REGULATION OF ADULT REPRODUCTIVE DIAPAUSE IN C. ELEGANS

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NIDDK

The hexosamine biosynthetic pathway uniquely integrates metabolites from several pathways essential to increasing lifespan, including glucose signaling and fatty acid metabolism. The enzyme *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) transferase (OGT) catalyzes the addition of this monosaccharide onto numerous intracellular proteins, which can subsequently be removed by *O*-GlcNAcase (OGA). Intriguingly, many of the proteins that are known to be essential in distinct pro-longevity pathways either interact with the *O*-GlcNAc cycling enzymes or are substrates. Thus, *O*-GlcNAc cycling is uniquely positioned at the center of numerous pro-longevity pathways and may act to integrate and disseminate distinct signals depending on the nature of the nutrient flux.

One powerful pro-longevity model, adult reproductive diapause (ARD), was recently discovered in *C. elegans*. During ARD, dietary restriction leads to lifespan extension as well as lengthened reproductive potential, via shrinkage and subsequent regeneration of the germline. Therefore, this model allows for the simultaneous analysis of distinct pro-longevity pathways and supports the theory that there are direct links between nutrient status, germline dynamics, and longevity.

We have discovered that loss of *O*-GlcNAc cycling in *C. elegans* significantly disrupts ARD entry. Coupled with this disruption, we observed that there are changes in the fatty acid composition and localization in mutants, suggesting a significant metabolic shift underlies this phenotype. These findings suggest that perturbation of nutrient-sensitive *O*-GlcNAc cycling is crucial to the regulation of entry into adult reproductive diapause. Understanding ARD dynamics is important as it provides a unique model in which to explore the relationship between nutrient status and the regulation of reproduction and aging.

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OGT REGULATES VASCULAR CELL PROLIFERATION AND GLUCOSE UTILIZATION IN IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION

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Background—Metabolic dysregulation has emerged as a major area of research in the pathobiology of idiopathic pulmonary arterial hypertension (IPAH). We recently published that increased O-linked N-acetyl-glucosamine (O-GlcNAc) transferase (OGT) was shown to enhance pulmonary arterial smooth muscle cell (PASMC) proliferation and worsen IPAH disease outcomes. OGT is a nutrient 'sensor' and is involved in cell cycle and signaling, proliferation, and metabolism. Proper homeostasis of the OGT/O-GlcNAc axis is required for proper cell function. An imbalance in the axis can 'drive' disease progression by altering cell proliferation and nutrient metabolism.

Methods—Human IPAH and control patient PASMCs were subjected to glucose bioenergetics analysis using an extracellular flux analyzer (Seahorse Bioscience) with and without OGT inhibitor. The rates of glycolysis, capacity, and reserve were calculated for these experiments. In addition, TCA cycle intermediates were examined by LC/MS/MS upon gene silencing of OGT in IPAH PASMCs.

Results—Basal glycolytic rates were lower in IPAH PASMCs than controls, but were not significantly different. However, glycolytic capacity and reserve rates were increased in IPAH PASMCs compared to controls [ECAR (mpH/min): (IPAH: 34.4±3.6; control: 19.2±4.8, $p<0.01$) and (IPAH: 25.4±2.7, control: -0.3±5.8, $p<0.01$), respectively]. Upon treatment with an OGT inhibitor, glycolytic capacity and reserve rates in IPAH PASMCs were reduced similar to control PASMCs levels [ECAR (mpH/min); (IPAH with OGT inhibitor: 13.9±1.3; IPAH: 34.4±3.6; control: 19.2±4.8, $p<0.001$) and (IPAH with OGT inhibitor: 2.9±3.4; IPAH: 25.4±2.7; control: -0.3±5.8, $p<0.001$, respectively]. Consistent with this, metabolomics studies demonstrated that key tricarboxylic acid cycle intermediates (succinate, a-ketoglutarate, and citrate), that are driven by oxidative phosphorylation, increased upon gene-silencing of OGT by siRNA in IPAH PASMCs [(succinate (ng/µl): IPAH with OGT siRNA: 5.4±0.1; IPAH: 3.4±0.3, $p<0.001$); (a-ketoglutarate (ng/µl): IPAH with OGT siRNA: 0.9±0.1; IPAH: 0.5±0.2, $p<0.05$); and (citrate (ng/µl): IPAH with OGT siRNA: 13.4±2.0; IPAH: 8.7±0.9, $p<0.05$)].

Conclusions—Our data demonstrate that modulation of OGT activity or expression in IPAH PASMCs results in changes in cell proliferation, glucose utilization, and energy production consistent with the Warburg effect phenomenon. We believe that the OGT/O-GlcNAc axis is a major regulator of the glucose 'metabolic switch' in IPAH PASMCs (similar to cancer cells).

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IDENTIFICATION AND LABEL-FREE QUANTIFICATION OF O-GLCNAC SITES IN ACTIVATED HUMAN T CELLS

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Glycosylation of intracellular proteins at serine/threonine residues by O-linked N-acetylglucosamine (O-GlcNAc) is now appreciated as an important post-translational modification with regulatory implications. Recently, we observed that activation of primary human T cells with anti-CD3/CD28 leads to elevated levels of O-GlcNAc glycosylation, and in the absence of O-GlcNAc transferase activity, T cell proliferation and cytokine production is impaired. While thousands of proteins have been identified as O-GlcNAc-modified, including hundreds in T cells, the modification sites of most proteins remain elusive, hindering mechanistic studies of how O-GlcNAc regulates protein function and quantitative analysis of O-GlcNAc sites between different cellular states. To address these issues, we applied Isotope Targeted Glycoproteomics (IsoTaG), an isotope-directed chemical glycoproteomics platform for characterization of metabolically labeled glycopeptides in a site-specific manner, to analyze O-GlcNAc glycosylation in resting and activated primary human T cells using less than 500 µg protein per condition. T cells were labeled with N-azidoacetyl glucosamine (O-GlcNAz), a metabolic reporter for O-GlcNAc, and then activated with anti-CD3/CD28 or pharmacological agonists for 18 hrs. Chemical enrichment and mass-independent mass spectrometry using electron transfer dissociation and high and low energy collision-induced dissociation fragmentation methods resulted in the identification of hundreds of glycopeptides, many of which represent novel glycoproteins and glycosites. Furthermore, this glycosite-specific approach allowed quantitative comparison of O-GlcNAc between resting and activated T cells. Glycosylation of several transcription factors, including NFAT and members of the JUN family, appears to be increased in activated T cells, suggesting that these O-GlcNAc sites may hold functional significance. Our results demonstrate that IsoTaG is an effective method for the unbiased identification and quantification of O-GlcNAc-modified glycopeptides from limited sample quantities. Future work will focus on how the identified O-GlcNAc sites, especially those with higher abundance in activated T cells, influence protein function and T cell activation.

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AN INTEGRATED SOLUTION FOR HIGH-THROUGHPUT, USER-FRIENDLY GLYCOANALYSIS USING RAPID SEPARATION BY CAPILLARY ELECTROPHORESIS

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ProZyme, Inc.

Glycan characterization is becoming necessary in the earliest stages of biotherapeutic cell line development, to the point where cell culture screening often requires glycan profiling. This entails significantly increased throughput for sample preparation, analytical instrumentation, data processing and expertise in glycan characterization. Unfortunately, these factors can cause a bottleneck to results. Here we present a glycan analysis solution that provides rapid sample preparation and analysis combined with a simplified data processing approach, enabling relative N-glycan quantification for 96 cell culture samples within a single workday.

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STRUCTURAL SNAPSHOTS OF NOTCH XYLOSYLATION SUPPORT AN SNI-LIKE RETAINING MECHANISM

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Notch signaling, a key pathway in animal development, is modulated by differential O-linked glycosylation of the epidermal growth factor-like (EGF) repeats of the Notch extracellular domain (NECD). Xyloside xylosyltransferase 1 (Xxylt1) adds terminal xylose to form an O-linked Xylosea1-3Xylosea1-3Glucose trisaccharide on Notch EGF repeats and negatively regulates Notch activation. Xxylt1 retains the anomeric configuration during its catalysis reaction and the underlying retaining mechanism is a major remaining question in glycobiology. In this study, using natural acceptor and donor ligands and active Xxylt1, we report a series of structural snapshots of the catalysis pathway of Xxylt1, including an unprecedented competent Michaelis complex for retaining GTs, its subsequent product complex and a Michaelis complex with un-favored donor ligand. The precise reaction geometry obtained from these complexes strongly supports a substrate-assisted S_Ni-like retaining catalytic mode where Xxylt1 functions to orient ligands instead of contributing nucleophile. Moreover, the use of folded disaccharide-modified EGF as acceptor in our structural research reveals an unexpected dramatic conformational change of EGF module, enabling the disaccharide to insert into the Xxylt1 catalytic pocket to reach the donor ligand. Finally, we analyze the Xxylt1 alterations in cancers and find that *XXYLT1* is frequently amplified in a subset of cancers, notably including lung squamous cell carcinoma. This suggests a potential pathogenic role of this enzyme in specific cancer types. Our mechanistic and functional insights into Xxylt1 will improve the understanding of the elusive retaining mechanism and promote the development of Xxylt1 inhibitors as potential therapeutics.

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ANOTHER FUNCTION OF N-LINKED GLYCOSYLATION: PREVENTING DEAMIDATION IN GLYCOPROTEINS AND GLYCOPEPTIDES

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In this work, preventing deamidation was described as a new function of *N*-linked glycosylation in glycoproteins and glycopeptides. Deamidation reaction where an Asparagine (Asn) in a protein is hydrolyzed to an Aspartic acid (Asp) is considered as an intrinsic gene-determined molecule clock to control life span of organisms through affecting functions of proteins. Protein deamidation poses a major problem for storage and quality control of some protein pharmaceuticals. We hypothesize that when *N*-linked glycosylation happens on the Asparagine residue, the deamidation on this residue should be greatly slowed. As a result, the half-life of the glycoprotein could be elongated significantly. To test our hypothesis, glycopeptides with a relatively small residue at the N+1 positions were selected to represent human glycoproteins that are susceptible to deamidation without glycosylation. Glycopeptides and corresponding non-glycosylated peptides were synthesized through solid phase peptide synthesizer (SPPS). In order to identify deamidation products, corresponding peptide products with Asn substituted by Asp and isoAsp were synthesized as standards. All of the non-glycosylated peptides have different half-lives ranging from one to twenty days through incubation under 37 °C at pH = 7.5. In our test periods, deamidation reaction was significantly reduced by *N*-linked GlcNAc. Glycopeptides with large natural N-glycans was also tested to as further proof of their preventative effects on deamidation. Furthermore, we applied this new function of N-linked glycosylation to a peptide drug candidate. Our results shows its on-shelf half-life was prolonged as expected.

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HOMOGENEOUS HEPARAN SULFATE OLIGOMERS FOR NMR STUDIES

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The extraordinary structural diversity of glycosaminoglycans (GAGs) enables them to interact with a wide variety of biological molecules to modulate processes, including immune response and regulation of cell growth. They are also involved in microbial pathogenesis, but this normally harmful process can be turned to advantage, as in the use of oncolytic viruses in cancer treatment. For these applications there is obvious interest in improving targeting to surface receptors up-regulated in cancer, including GAGs.

NMR methods provide means of structurally characterizing both GAG and protein components. In order to determine the structural basis of GAG-protein binding, it is necessary to use homogeneous HS oligomers, and it is helpful to be able to incorporate isotopic labeling of HS. Towards this goal, we have developed expression protocols for glypican (a glycoprotein with linked HS chains) in order to yield 15N-HS with specific sulfation patterns. Subsequent heparanase digestion and size-exclusion chromatography yields oligomers homogeneous in both sulfation pattern and length.

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RAPID-THROUGHPUT GLYCOMICS TO DISCOVER THE ROLE OF HUMAN MILK OLIGOSACCHARIDES IN INFANT HEALTH

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Oligosaccharides are diverse structures that have proven difficult to analyze due to their composition complexity. Analysis is complicated further when profiles of hundreds of samples are necessary for a single study. Human milk oligosaccharides (HMOs) are composed of five monosaccharides and can either be branched or linear, decorated or undecorated. Although indigestible by the infant, HMOs act as prebiotics and probiotics to promote a healthy digestive tract.

A rapid-throughput HMO extraction method was used to isolate and purify HMOs in studies composed of hundreds of samples in order to correlate mother's HMO profile to her infant's health and growth outcomes. If the extraction was performed on multiple plates in multiple batches we accounted for fluctuations in sample preparation by including one sample in triplicate on all plates. Absolute abundances were normalized to an external HMO pool injected intermittently throughout the run to account for batch to batch instrument fluctuations. Programming scripts to sum volumes of HMO class abundances and align peaks for structural identification were created in-house to aid in high-throughput data analysis. The phenotypic secretor status of each mother was determined by calculating the relative abundance of several α1-2 linked fucose containing structures found consistently in all samples.

This method was applied to examine 33 mother-infant pairs from the Gambia. Milk samples from the mothers and anthropometric and morbidity data of the infants were taken at 4, 16, and 20 weeks postpartum. We discovered that infants who did not have sick days received more MFLNH I and III over time, which was the opposite trend for infants who did get sick. Infants with abnormal levels of stool calprotectin, and intestinal inflammation marker, were shorter than infants with normal levels, and infants with normal levels received milk with increasing amounts of α1-2 fucosylated HMO and decreasing amounts of sialylated HMO. Total fucosylation was found to be a good predictor of weight-for-age Z scores at 20 weeks.

These results demonstrate that fucosylation is beneficial in preventing infection and promoting growth, and that our rapid and high-throughput method can aid in determining the health implications of various HMO structures.

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PPGALNAC-T13 WAS REQUIRED FOR STABILIZING MUCIN-TYPE O-GLYCOPROTEIN PODOPLANIN DURING P19 CELL NEURONAL DIFFERENTIATION

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Cell-surface protein glycosylation is essential for the viability, function and differentiation of neural cells. Mucin-type O-glycosylation in nervous system remains largely unknown. The mucin-type O-glycosylation is initiated by a large enzyme family of polypeptide N-acetylgalactosaminyltransferase (ppGalNAc-Ts). ppGalNac-T13 (T13) is restrictively and highly expressed in neuronal cells, the role and substrate of T13 in neuronal differentiation are still unknown. Here, using a neuronal differentiation cell model based on mouse embryonal carcinoma P19 cells, we found T13 was an ER-localized protein and was upregulated during neuronal differentiation, whereas CRISPR-Cas9 mediated knockout of T13 suppressed ATRA (all-trans retinoic acid) induced P19 cell aggregation and delayed neuronal differentiation in this model. As a substrate candidate of T13, we found that Podoplanin (PDPN) expression and distribution patterns were consistent to T13 during neuronal differentiation detected by western blotting and immunofluorescence. PDPN has been found to be expressed in mouse central nervous system, it is a mucin-type O-glycoprotein and its classical function was to stimulate platelet aggregation and activation by interacting with CLEC-2. Interestingly, our analysis for T13 knockout P19 cells undergoing neuronal differentiation and in vitro catalytic reaction results indicated that T13 could glycosylate PDPN, while deficiency of O-GalNAcylation significantly reduced the half-life of PDPN in CHO ldlD cells and T13 knockout P19 cells. Those findings suggested that increased expression of T13 contribute to P19 cells neuronal differentiation via glycosylating and stabilizing PDPN.

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DEVELOPMENT OF A RAPID HILIC-UPLC METHOD FOR GLYCAN ANALYSIS OF RECOMBINANT PROTEINS

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Biogen

Glycosylation has been demonstrated to play important roles in many biological processes, including function, pharmacokinetics, pharmacodynamics, stability, and immunogenicity of proteins. Thus, it is critical to monitor recombinant protein glycan heterogeneity to ensure safety, efficacy and manufacturing consistency of biopharmaceuticals. One disadvantage of currently widely used glycan analysis method is the long sample preparation process, which includes overnight N-glycan release, protein removal, 2-aminobenzamide (2-AB) labeling and often an additional purification to remove salts and an excess of label reagents. The entire sample preparation process takes up to three days. Another disadvantage of using 2-AB dye for glycan labeling is the low ionization efficiency in liquid chromatography with mass spectrometry (LC-MS) analysis, which limits the structure identification of complex glycans.

In this study, the glycan analysis of highly sialylated recombinant protein was evaluated between traditional, overnight enzymatic glycan release assay as described above and a novel *Rapi*Flour kit. In later approach, experimental conditions of glycan release including denature time, denature temperature, surfactant amount and incubation time were optimized resulting in total sample preparation time of less than two hours. Using the optimized conditions for *Rapi*Flour kit, the glycan profiles and relative amounts of detected glycan between two methods were comparable. In addition, the use of new labeling dye from *Rapi*Flour kit enhances ionization efficiency of glycan detection by mass spectrometry (MS), which facilitates the glycan structure identification compared to 2-AB dye.

Details around assay optimization of novel *Rapi*Flour kit for analysis of complex glycans by HILIC with fluorescence detection and HILIC-MS will be described.

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A VERSATILE, HIGH-PERFORMANCE MICROARRAY DISPLAYING SITE-SPECIFICALLY GLYCOSYLATED MUCIN GLYCOPEPTIDES TO DETECT ANTIBODIES DIRECTED TO DISEASE-RELEVANT CONFORMATIONAL EPITOPES

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Microarrays displaying a wide range of glycans have facilitated the understanding of the binding preferences of lectins and related molecules. Furthermore, the identification of MUC1 tumor-associated Tn antigen (GalNAc□1-O-Ser/Thr) suggests that a structurally defined glycopeptide-imprinted microarray would be of great interest for biomarker discovery research and would impact the development of potential anticancer vaccines. Such microarrays displaying antigenic glycopeptides is an attractive and powerful tool for high-throughput screening of antibodies and its epitope mapping analysis including glycan-binding profiles.

We have been concerned with an efficient and versatile method for the preparation of microarrays displaying mucin-type glycopeptides to accelerate a creation of a new class of diagnostic and therapeutic anti-mucin monoclonal antibodies with a promising clinical outcome. In the present study, we report a facile and standardized protocol for the fabrication of high-performance microarrays displaying a MUC1-related peptides/glycopeptides library by means of the "glycoblotting" method, a general protocol for the site-regulated immobilization of compounds with an aldehyde or an equivalent ketone functional group under a mild aqueous condition without use of any coupling reagent. Employing an aminooxy-functionalized methacrylic copolymer with phosphorylcholine unit, selective imine-coupling of a series of synthetic MUC1 glycopeptides capped by a ketone linker at their *N*-terminus provided a straightforward and seamless protocol for the preparation of the MUC1 glycopeptide microarray platform. The resulting microarray platform enabled highly sensitive and accurate epitope mapping analysis with a significantly reduced background noise level due to the phosphorylcholine unit in the array surface. Advantages of our array format demonstrated that anti-KL-6 monoclonal antibody shows an extremely specific and strong binding affinity toward MUC1 fragments carrying sialyl T antigen (Neu5Ac□2,3Gal□1,3GalNAc□1→) at Pro-Asp-**Thr**-Arg motif when compared with the other seven anti-MUC1 monoclonal antibodies such as VU-3D1, VU-12E1, VU-11E2, Ma552, VU-3C6, SM3, and DF3. The present microarray also uncovered the occurrence of IgG autoantibodies in healthy human sera that bind specifically with sialyl T antigen attached at five potential *O*-glycosylation sites of MUC1 tandem repeats.

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ABERRANT O-GLYCOSYLATION AFFECTS THE TRANSCRIPTIONAL RESPONSE DOWNSTREAM OF MUC1 AND EGFR IN BREAST CANCER

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Aberrant glycosylation occurs in essentially all types of human cancer and appears to be an early event, as well as playing a key role in the induction of invasion and metastases. In breast cancer, 90% of tumours show changes in the composition of the O-glycans carried by glycoproteins. A switch often observed is the transition from elongated core-2 based O-glycans to short sialylated core-1 based O-glycans. Nevertheless little is known about how these changes affect the function of individual proteins that are key to the transformation and metastatic process. We have therefore investigated how changes in O-glycosylation affect the function of two O-linked glycoproteins, MUC1 and EGFR in breast cancer.

MUC1 is a transmembrane mucin that is heavily O-glycosylated and has been shown to interact with EGFR. EGFR is a membrane receptor that upon binding of EGF is phosphorylated and activates signaling pathways that lead to cell growth, resistance to apoptosis and cell motility. Moreover EGFR itself can translocate to the nucleus where it binds to several gene promoters during which time it interacts with the cytoplasmic tail of MUC1.

Using a breast carcinoma cell line expressing sialylated core-1 O-glycans and an isogenic cell line expressing core-2 O-glycans, genome wide transcriptional changes in response to EGF stimulation were compared. The two cell lines clearly show a different gene expression signature confirming that the O-linked glycosylation status of the cell influences gene expression. Analysis of the arrays shows that many of the genes differentially expressed between the two cell lines encode for proteins known to regulate cell division and the tumour microenvironment.

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SOLID-PHASE EXTRACTION OF N-LINKED GLYCANS AND GLYCOSITE-CONTAINING PEPTIDES (NGAG) FOR COMPREHENSIVE CHARACTERIZATION OF GLYCOPROTEINS

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Protein glycosylation plays fundamental roles in biological processes and pathological progression of diseases. Glycoproteins modified by N-linked oligosaccharides are complex with each glycoprotein may potentially containing multiple glycosylation sites and each glycosylation site consisting of heterogeneous glycoforms. Comprehensive characterization of protein glycosylation is critical to understanding the structures and functions of glycoproteins. However, due to the enormous complexity and heterogeneity of glycoprotein structures, the current glycoprotein analyses focus mainly on either released glycans or de-glycosylated glycosite-containing peptides. In this study, we describe a novel chemoenzymatic method called solid phase extraction of N-linked Glycans And Glycosite-containing peptides (NGAG) for the comprehensive characterization of glycoproteins by the simultaneous analysis of N-linked glycans, glycosite-containing peptides, glycoproteins, and glycans attached to specific glycosites via intact glycopeptides. We show that the NGAG method allowed us to identify 85 N-linked glycan compositions, 2,044 glycosite-containing peptides, 1, 242 glycoproteins and 1,562 intact glycopeptides from an ovarian cancer cell line (OVCAR-3). The NGAG method can also be applied to quantitatively determine glycoprotein alterations in glycosite occupancy as well as total and site-specific glycans. This method opens up new avenues for the large-scale comprehensive characterization of glycoproteins.

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GPQUEST: A SOFTWARE TOOL FOR GLYCOPROTEOMICS ANALYSIS OF COMPLEX BIOLOGICAL SAMPLES

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Glycoproteomics, the high-throughput study of site-specific protein glycosylation using mass spectrometry techniques, is a rapidly growing field. Recent advances in instrumentation, particularly the emergence of Orbitrap mass analyzer and overcoming of the ion trap low mass cut off has facilitated the analysis of intact glycopeptides, which is essential for preserving the glycosylation microheterogeneity information of samples. On one hand, massive amount of data is being generated at a rate of 10GB/hour on a single instrument. On the other hand, with the software and analysis tools lagging behind, this data is not being fully interrogated. Therefore, there is a great need for development of analysis tools that can dig the glycoproteomics data. Here we report a software tool for identification of intact glycopeptides from simple and complex biological samples, such as recombinant glycoprotein cocktails and cell, serum or tissue samples. GPQuest has an easy to use graphical user interface and is available for Macintosh and Windows 64-bit systems. Two algorithms, including "Precursor Mass Matching" and "Spectral Library Matching", are implemented in GPQuest, which offer the advantage of lower execution time and more identification, respectively. In addition, the user is able to extract the intensity of any reporter ions of interest, such as iTRAQ, oxonium or custom reporter ions. The interface provides the user with options to narrow down the peptide-spectral matches based on the number of present oxonium ions, matching b and y ions, and intact peptide ions with partial glycans. In addition, a pseudo-morpheus score is assigned to each peptide-spectral match and a decoy strategy is employed to provide statistics on the false discovery rate. GPQuest has been tested on several databases including, gp120 HIV envelop recombinant glycoprotein, prostate (LNCAP and PC3) and ovarian (OVCAR3) cancer cells and serum samples and the matches have been checked manually for verification. GPQuest is one of the first glycoproteomics software tools of many that can provide insight into the microheterogeneity of glycosylation in biological and clinical samples by filling some of the gaps left in proteomic-only analysis workflows.

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DISRUPTION OF GOLGI MORPHOLOGY AND ALTERED PROTEIN GLYCOSYLATION IN PLA2G6-ASSOCIATED NEURODEGENERATION

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Background: Mutations in *PLA2G6*, which encodes the calcium-independent phospholipase A2 group VI, cause neurodegeneration and diffuse cortical Lewy body formation by a yet undefined mechanism. We assessed whether altered protein glycosylation due to abnormal Golgi morphology might be a factor in the pathology of this disease.

Methods: Three patients presented with *PLA2G6*-associated neurodegeneration (PLAN); two had infantile neuroaxonal dystrophy (INAD) and one had adult-onset dystonia-parkinsonism. We analyzed protein N-linked and O-linked glycosylation in cerebrospinal fluid, plasma, urine, and cultured skin fibroblasts using HPLC and MALDI-TOF/MS. We also assessed sialylation and Golgi morphology in cultured fibroblasts by immunofluorescence and performed rescue experiments using a lentiviral vector.

Results: The patients with INAD had *PLA2G6* mutations NM_003560.2: c.[950G>T];[426-1077dup] and c.[1799G>A];[2221C>T] and the patient with dystonia-parkinsonism had *PLA2G6* mutations NM_003560.2: c.[609G>A];[2222G>A]. All three patients had altered Golgi morphology and abnormalities of protein O-linked glycosylation and sialylation in cultured fibroblasts that were rescued by lentiviral overexpression of wild type PLA2G6.

Conclusions: Our findings add altered Golgi morphology, O-linked glycosylation and sialylation defects to the phenotypic spectrum of PLAN; these pathways are essential for correct processing and distribution of proteins. Lewy body and Tau pathology, two neuropathological features of PLAN, could emerge from these defects. Therefore, Golgi morphology, O-linked glycosylation and sialylation may play a role in the pathogenesis of PLAN and perhaps other neurodegenerative disorders

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AN AGNOSTIC SCREEN OF PROTEIN GLYCOSYLATION IN THE ATTEMPT TO DIAGNOSE RARE GENETIC DISORDERS AND UNRAVEL MECHANISM OF DISEASE

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The NIH Undiagnosed Diseases Program(UDP) concentrates its efforts on the most puzzling medical cases, focusing on disease discovery and patient diagnosis using both genomic data and basic research. Some UDP patients could have Congenital Disorders of Glycosylation (CDGs), whose phenotypes lack consistent clinical features or biomarkers, and are difficult to diagnose. Therefore, we undertook an agnostic screen of plasma and urine to identify abnormalities of protein glycosylation in UDP patients.

Plasma and urine screens were completed for 138 patients, and included profiling of plasma N-linked glycans by MALDI-TOF, plasma O-linked glycans by LC-MS/MS and MALDI-TOF, and urine free oligosaccharides by MALDI-TOF/TOF. Over 65% of UDP patients had a glycosylation profile that deviated from a healthy control population in at least one specimen. Additional evaluation of the N-linked and O-linked glycosylation profiles in primary dermal fibroblasts of 59 patients with glycome abnormalities resulted in a tractable glycome phenotype in ~50% of these cases.

Using this approach, we identified different CDGs, lysosomal storage disorders (affecting glycoprotein degradation), and rare disorders in which glycosylation is affected indirectly. We also added abnormal glycosylation findings to the phenotypical spectrum of a known rare disease, shedding light on a potential factor of the underlying pathogenic mechanism. In summary, we describe a comprehensive screening approach to identify primary protein glycosylation abnormalities and provide a useful tool for biochemically phenotyping patients with unknown diseases and unraveling the underlying mechanisms of diseases.

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TOXOPLASMA GONDII MICRONEME PROTEINS 1 AND 4 RECOGNIZE N-GLYCANS OF TLRS: (1) TGMIC1 AND TGMIC4 INTERACT WITH TLR2 N-GLYCANS

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Several proteins of the protozoan Toxoplasma gondii are preassembled in the endoplasmic reticulum and form complexes prior to transit towards apical organelles, such as micronemes, whose content is released during the invasion process of host cells. Among these adhesive complexes, the multimeric one formed by TgMIC6, TgMIC1, and TgMIC4 contributes to the parasite virulence. The N-terminus of TgMIC1 contains two micronemal adhesive repeats (MAR) that bind sialic acid, whereas TgMIC4 has two tandem apple domains that bind galactose-containing glycans. Beyond promoting adhesion to host cells, recombinant forms of TgMIC1 and TgMIC4 stimulate macrophages to produce proinflammatory cytokines, which are known to induce protective immunity against the parasite. This stimulatory activity was inhibited when macrophages from TLR2-/- and TLR4-/- mice were assayed. In the current study we investigate the direct interaction of rTgMIC1 and rTgMIC4 with TLR2 N-glycans. We used HEK293T cells that were transfected with plasmids containing the sequences either for TLR2 wild-type or for TLR2 glycomutants ectodomains, in order to express the receptor lacking in one or more N-glycosylation sites. We found that the HEK293T cell activation, manifested by IL-8 production, was significantly reduced in response to stimulation with rTgMIC1 and rTgMIC4, when the glycomutants were transfected, in comparison to wild-type TLR2. We could identify that, among the four glycans N-linked to TLR2, rTgMIC1 recognizes those occupying the second, third and fourth positions, whereas rTgMIC4 recognizes the N-glycan occupying the fourth position. This work clearly demonstrates the occurrence of interaction of TgMIC1 and TgMIC4 with TLR2 N-linked glycans and contributes to better understanding the role performed by micronemes proteins of *T. gondii* on host cells.

Keywords: *Toxoplasma gondii,* microneme proteins, toll-like receptors, carbohydrate recognition
Financial support: FAPESP, CNPq

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TOXOPLASMA GONDII MICRONEME PROTEINS 1 AND 4 RECOGNIZE N-GLYCANS OF TLRS: (2) THE INTERACTION TRIGGERS THE INITIAL IMMUNE RESPONSE TO THE PROTOZOAN

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Toxoplasma gondii actively infect host cells through a dependent process of lectins release, called microneme proteins (MIC), from intracellular organelles. TgMIC1, TgMIC4 and TgMIC6 assembly a complex on *T. gondii* surface enabling the parasite to bind to host cells via carbohydrate recognition, since TgMIC1 binds to terminal sialic acid and TgMIC4 to terminal galactose. Our aim was to evaluate the carbohydrate-protein interaction between TgMIC1 and TgMIC4 with *N*-glycans of extracellular TLRs and the innate immune response triggered by this interaction. We observed that TgMIC1 and TgMIC4 (native complex and recombinant proteins) induced proinflammatory cytokines production, especially IL-12, by C57BL/6 mice dendritic cells (DCs) and macrophages similarly to TLR agonists, while TLR2-/-, TLR4-/- or DKO-TLR2-/-/TLR4-/- macrophages produced less IL-12 than wild type (WT) cells. Furthermore, we found that this activation was dependent on carbohydrate recognition since a punctual mutation in carbohydrate recognition domain (CRD) of TgMIC1 abrogated its capacity to induce IL-12 production by WT macrophages. Moreover, during the first hours of *T. gondii* infection, the absence of TLR2 and TLR4 resulted in lower IL-12 production by DCs. Finally, the infection of WT DCs and macrophages with parasites lacking TgMIC1 or both proteins (DKO-TgMIC1-/-/TgMIC4-/-) also resulted in impaired activation, indicated by lower IL-12 production, compared to infection with WT parasites. Since the decreased IL-12 production was observed in the first hours after infection, we found that TgMIC1 and TgMIC4 trigger the initial response to *T. gondii*, by recognizing *N*-glycans of TLRs. The established interactions and the resulting host cell activation may exert relevant biological role during *T. gondii* infection.

Keywords: *Toxoplasma gondii*, microneme proteins, toll-like receptors, carbohydrate recognition
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TOXOPLASMA GONDII MICRONEME PROTEINS 1 AND 4 RECOGNIZE N-GLYCANS OF TLRS: (2) THE INTERACTION TRIGGERS THE INITIAL IMMUNE RESPONSE TO THE PROTOZOAN

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As an immune evasion strategy, Plasmodium *falciparum* malaria parasites modify infected erythrocytes (IEs) to present a unique surface protein called VAR2CSA. This protein allows the IEs to adhere to a distinct type of chondroitin sulfate (CS) exclusively expressed in the placenta. In the placenta CS promotes cellular proliferation and tissue invasion as part of the normal placental implementation process. Rapid proliferation and cellular migration are features shared with cancer cells. We show that placental-like CS is present on a high proportion of malignant cells and tissues, while being absent from normal tissue, and that it can be specifically targeted by recombinant VAR2CSA (rVAR2). In tumors, placental-like CS chains are linked to a limited repertoire of cancer-associated proteoglycans including CD44 and CSPG4. High placental-like CS expression correlates with progression of malignant melanoma and predicts poor relapse-free survival in non-small cell lung cancer patients. The rVAR2 protein localizes to tumors *in vivo* and rVAR2 fused to diphtheria toxin or conjugated to hemiasterlin compounds strongly inhibits *in vivo* tumor cell growth and metastasis. This work demonstrates how an evolutionarily refined parasite-derived protein can be exploited to target a common, but complex, malignancy-associated glycosaminoglycan modification. The data confirms the pivotal role of placental-type CS in cancer and supports the use of rVAR2 as a cancer specific targeting reagent for therapy and diagnosis.