A glycan-based approach to cell characterization and isolation: Hematopoiesis as a paradigm

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- J Exp Med
- https://pubmed.ncbi.nlm.nih.gov/36066492/

Cell surfaces display a wide array of molecules that confer identity. While flow cytometry and cluster of differentiation (CD) markers have revolutionized cell characterization and purification, functionally heterogeneous cellular subtypes remain unresolvable by the CD marker system alone. Using hematopoietic lineages as a paradigm, we leverage the extraordinary molecular diversity of heparan sulfate (HS) glycans to establish cellular "glycotypes" by utilizing a panel of anti-HS single-chain variable fragment antibodies (scFvs). Prospective sorting with anti-HS scFvs identifies functionally distinct glycotypes within heterogeneous pools of mouse and human hematopoietic progenitor cells and enables further stratification of immunophenotypically pure megakaryocyte-erythrocyte progenitors. This stratification correlates with expression of a heptad of HS-related genes that is reflective of the HS epitope recognized by specific anti-HS scFvs. While we show that HS glycootyping provides an orthogonal set of tools for resolution of hematopoietic lineages, we anticipate broad utility of this approach in defining and isolating novel, viable cell types across diverse tissues and species.

- Rationale: A new approach to cell fractionation based on HS glycootyping of stem cells and differentiation using hematopoiesis as a model.

O-linked α2,3 sialylation defines stem cell populations in breast cancer

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- Sci Adv
- https://pubmed.ncbi.nlm.nih.gov/34995107/

We pursued the hypothesis that specific glycans can be used to distinguish breast cancer stem cells (CSCs) and influence their function. Comparison of CSCs and non-CSCs from multiple breast cancer models revealed that CSCs are distinguished by expression of α2,3 sialylated core2 O-linked glycans. We identified a lectin, SLBR-N, which binds to O-linked α2,3 sialic acids, that was able to enrich for CSCs in vitro and in vivo. This O-glycan is expressed on CD44 and promotes its interaction with hyaluronic acid, facilitating CD44 signaling and
CSC properties. In contrast, FUT3, which contributes to sialyl Lewis X (sLeX) production, is preferentially expressed in the non-CSC population, and it antagonizes CSC function. Collectively, our data indicate that SLBR-N can be more efficient at enriching for CSCs than CD44 itself because its use avoids the issues of CD44 splicing and glycan status. These data also reveal how differential glycosylation influences CSC fate.

○ **Rationale:** Identification of cancer stem cells (cells responsible for initiation and recurrence of cancer) is still a challenge. Thus, I find it very interesting that this group decided to look into sugars to distinguish different cell populations and to also predict cell behavior and stem cell fate.

### 3. Membrane glycome is impacted by the cell culturing mode of neuroblastoma cells with differing migration and invasion potential

○ **Zeynep Sumer-Bayraktar 1, Christopher M Fife 2 3, Frances L Byrne 2, Maria Kavallaris 2 3 4, Nicolle H Packer 1 5**

○ **Glycobiology**

○ [https://pubmed.ncbi.nlm.nih.gov/35312763/](https://pubmed.ncbi.nlm.nih.gov/35312763/)

○ Neuroblastoma is a highly metastatic childhood cancer for which studies indicate an association between protein glycosylation and tumor behavior. However, there is a lack of detailed glycome analysis on neuroblastoma cells that have varying metastatic potential. Furthermore, the impact of the cell culturing mode, i.e. 2-dimensional (2D) versus 3-dimensional (3D) spheroids, on the membrane protein glycome is unknown. To address these gaps in knowledge, we mapped membrane protein N- and O-glycosylation of neuroblastoma cells that have lower invasive and metastatic potential (Stathmin shRNA-expressing cells, StmnSeq2SH, and StmnSeq3SH) compared with control cells (control shRNA-expressing cells, CtrlSH). We showed that the neuroblastoma cells with different migratory and invasive potential underwent drastic changes in their membrane protein N-glycosylation exclusively when cultured in 3D spheroids. We also investigated the impact of 2D and 3D cell culture methods on cellular glycosylation using the neuroblastoma cells and found the cell N-glycome was markedly impacted by the culture method, with the 2D grown cells showing an abundance of oligomannosidic glycans, whereas 3D spheroids expressed more complex type glycans on their membrane proteins. In summary, this study provides the first comprehensive protein glycome profiling of neuroblastoma cells that have varying invasiveness and migratory potential and unravels the distinct membrane glycan features of cells that are grown under 2D versus 3D culture conditions.

○ **Rationale:** I am definitely new in the field, so I find this pretty interesting because of my cell culture centric project. Honestly, I never even thought about it, but I
think that given the common use of cell culture in research the awareness of modification in the membrane glycoprotein landscape should be kept into consideration.

4. Revealing the human mucinome
   ○ Stacy A Malaker # 1 2, Nicholas M Riley # 3, D Judy Shon 3, Kayvon Pedram 3, Venkatesh Krishnan 4, Oliver Dorigo 4, Carolyn R Bertozzi 5 6
   ○ Nat Commun
   ○ https://pubmed.ncbi.nlm.nih.gov/35725833/
   ○ Mucin domains are densely O-glycosylated modular protein domains found in various extracellular and transmembrane proteins. Mucin-domain glycoproteins play important roles in many human diseases, such as cancer and cystic fibrosis, but the scope of the mucinome remains poorly defined. Recently, we characterized a bacterial O-glycoprotease, StcE, and demonstrated that an inactive point mutant retains binding selectivity for mucin-domain glycoproteins. In this work, we leverage inactive StcE to selectively enrich and identify mucin-domain glycoproteins from complex samples like cell lysate and crude ovarian cancer patient ascites fluid. Our enrichment strategy is further aided by an algorithm to assign confidence to mucin-domain glycoprotein identifications. This mucinomics platform facilitates detection of hundreds of glycopeptides from mucin domains and highly overlapping populations of mucin-domain glycoproteins from ovarian cancer patients. Ultimately, we demonstrate our mucinomics approach can reveal key molecular signatures of cancer from in vitro and ex vivo sources.
   ○ Rationale: Novel approach to identify ‘new’ mucins associated with cancers

5. Phase separation on cell surface facilitates bFGF signal transduction with heparan sulphate
   ○ Song Xue # 1 2, Fan Zhou # 1, Tian Zhao # 1, Huimin Zhao 1, Xuewei Wang 1, Long Chen 1, Jin-Ping Li 2 3, Shi-Zhong Luo 4
   ○ Nat Commun
   ○ https://pubmed.ncbi.nlm.nih.gov/35236856/
   ○ Liquid-liquid phase separation (LLPS) plays important roles in various cellular processes, facilitating membrane-less organelles construction, chromatin condensation, signal transduction on inner membrane and many other processes. Current perception is that LLPS relies on weak multivalent interactions and crowded environments intracellularly. In this study, we demonstrate that heparan sulfate can serve as a platform to induce the phase separation of basic fibroblast growth factor on cell surface. The phase separation model provides an alternative mechanism how bFGF is enriched to its receptors,
therefore triggering the signaling transduction. The research provides insights on
the mechanism how growth factors can be recruited to cell surface by heparan
sulfate and execute their functions, extending people’s view on phase separation
from intracellular to extracellular proteins at cellular level.

○ **Rationale:** Interesting alternate explanation for coreceptor activity of heparan
  sulfate

6. **A quartz crystal microbalance method to quantify the size of hyaluronan and
other glycosaminoglycans on surfaces.**

○ Sumitra Srimasorn # 1 2, Luke Souter # 1, Dixy E Green 3, Lynda Djerbal 1,
  Ashleigh Goodenough 1 2, James A Duncan 1 4, Abigail R E Roberts 1 2, Xiaoli
  Zhang 1 2, Delphine Débarre 5, Paul L DeAngelis 3, Jessica C F Kwok 6 7, Ralf
  P Richter 8 9

○ Sci Rep

○ [https://pubmed.ncbi.nlm.nih.gov/35768463/](https://pubmed.ncbi.nlm.nih.gov/35768463/)

○ Hyaluronan (HA) is a major component of peri- and extra-cellular matrices and
plays important roles in many biological processes such as cell adhesion,
proliferation and migration. The abundance, size distribution and presentation of
HA dictate its biological effects and are also useful indicators of pathologies and
disease progression. Methods to assess the molecular mass of free-floating HA
and other glycosaminoglycans (GAGs) are well established. In many biological
and technological settings, however, GAGs are displayed on surfaces, and
methods to obtain the size of surface-attached GAGs are lacking. Here, we
present a method to size HA that is end-attached to surfaces. The method is
based on the quartz crystal microbalance with dissipation monitoring (QCM-D)
and exploits that the softness and thickness of films of grafted HA increase with
HA size. These two quantities are sensitively reflected by the ratio of the
dissipation shift (ΔD) and the negative frequency shift (-Δf) measured by QCM-D
upon the formation of HA films. Using a series of size-defined HA preparations,
ranging in size from ~ 2 kDa tetrasaccharides to ~ 1 MDa polysaccharides, we
establish a monotonic yet non-linear standard curve of the ΔD/ - Δf ratio as a
function of HA size, which reflects the distinct conformations adopted by grafted
HA chains depending on their size and surface coverage. We demonstrate that
the standard curve can be used to determine the mean size of HA, as well as
other GAGs, such as chondroitin sulfate and heparan sulfate, of preparations of
previously unknown size in the range from 1 to 500 kDa, with a resolution of
better than 10%. For polydisperse samples, our analysis shows that the process
of surface-grafting preferentially selects smaller GAG chains, and thus reduces
the average size of GAGs that are immobilised on surfaces comparative to the
original solution sample. Our results establish a quantitative method to size HA
and other GAGs grafted on surfaces, and also highlight the importance of sizing GAGs directly on surfaces. The method should be useful for the development and quality control of GAG-based surface coatings in a wide range of research areas, from molecular interaction analysis to biomaterials coatings.

- **Rationale:** Interesting technique to measure the molecular mass of GAGs

7. **Origin of cytoplasmic GDP-fucose determines its contribution to glycosylation reactions**

- **Paulina Sosicka 1, Bobby G Ng 1, Lauren E Pepi 2, Asif Shajahan 2, Maurice Wong 3, David A Scott 4, Kenjiroo Matsumoto 2, Zhi-Jie Xia 1, Carlito B Lebrilla 3, Robert S Haltiwanger 2, Parastoo Azadi 2, Hudson H Freeze 1**

- **J Cell Biol**

- [https://pubmed.ncbi.nlm.nih.gov/36053214/](https://pubmed.ncbi.nlm.nih.gov/36053214/)

- Biosynthesis of macromolecules requires precursors such as sugars or amino acids, originating from exogenous/dietary sources, reutilization/salvage of degraded molecules, or de novo synthesis. Since these sources are assumed to contribute to one homogenous pool, their individual contributions are often overlooked. Protein glycosylation uses monosaccharides from all the above sources to produce nucleotide sugars required to assemble hundreds of distinct glycans. Here, we demonstrate that cells identify the origin/heritage of the monosaccharide, fucose, for glycosylation. We measured the contribution of GDP-fucose from each of these sources for glycan synthesis and found that different fucosyltransferases, individual glycoproteins, and linkage-specific fucose residues identify and select different GDP-fucose pools dependent on their heritage. This supports the hypothesis that GDP-fucose exists in multiple, distinct pools, not as a single homogenous pool. The selection is tightly regulated since the overall pool size remains constant. We present novel perspectives on monosaccharide metabolism, which may have a general applicability.

- **Rationale:** Interesting paper showing that there are subpools of nucleotide sugars in cells.

8. **N-glycolyneuraminic acid serum biomarker levels are elevated in breast cancer patients at all stages of disease**

- **Lucy K Shewell # 1, Christopher J Day # 1, Jamie R Kutasovic 2, Jodie L Abrahams 1 3, Jing Wang 1, Jessica Poole 1, Colleen Niland 2, Kaltin Ferguson 2, Jodi M Saunus 2, Sunil R Lakhani 2 4, Mark von Itzstein 1, James C Paton 5, Adrienne W Paton 5, Michael P Jennings 6**

- **BMC Cancer**

- [https://pubmed.ncbi.nlm.nih.gov/35346112/](https://pubmed.ncbi.nlm.nih.gov/35346112/)
Background: Normal human tissues do not express glycans terminating with the sialic acid N-glycolyneuraminic acid (Neu5Gc), yet Neu5Gc-containing glycans have been consistently found in human tumor tissues, cells and secretions and have been proposed as a cancer biomarker. We engineered a Neu5Gc-specific lectin called SubB2M, and previously reported elevated Neu5Gc biomarkers in serum from ovarian cancer patients using a Surface Plasmon Resonance (SPR)-based assay. Here we report an optimized SubB2M SPR-based assay and use this new assay to analyse sera from breast cancer patients for Neu5Gc levels. Methods: To enhance specificity of our SPR-based assay, we included a non-sialic acid binding version of SubB, SubBA12, to control for any non-specific binding to SubB2M, which improved discrimination of cancer-free controls from early-stage ovarian cancer. We analysed 96 serum samples from breast cancer patients at all stages of disease compared to 22 cancer-free controls using our optimized SubB2M-A12-SPR assay. We also analysed a collection of serum samples collected at 6 monthly intervals from breast cancer patients at high risk for disease recurrence or spread. Results: Analysis of sera from breast cancer cases revealed significantly elevated levels of Neu5Gc biomarkers at all stages of breast cancer. We show that Neu5Gc serum biomarker levels can discriminate breast cancer patients from cancer-free individuals with 98.96% sensitivity and 100% specificity. Analysis of serum collected prospectively, post-diagnosis, from breast cancer patients at high risk for disease recurrence showed a trend for a decrease in Neu5Gc levels immediately following treatment for those in remission. Conclusions: Neu5Gc serum biomarkers are a promising new tool for early detection and disease monitoring for breast cancer that may complement current imaging- and biopsy-based approaches.

Rationale: Clinically speaking, Neu5Gc serum biomarkers may be a promising new tool for early detection and disease monitoring for breast cancer. Worthy of discussion.

9. Lysosomal enzyme trafficking factor LYSET enables nutritional usage of extracellular proteins

Catarina Pechincha, Sven Groessl, Robert Kalis, Melanie de Almeida, Andrea Zanotti, Marten Wittmann, Martin Schneider, Rafael P de Campos, Sarah Rieser, Marlene Brandstetter, Alexander Schleiffer, Karin Müller-Decker, Dominic Helm, Sabrina Jabs, David Haselbach, Marius K Lemberg, Johannes Zuber, Sabrina Jabs, Wilhelm Palm

Science

https://pubmed.ncbi.nlm.nih.gov/36074822/

Mammalian cells can generate amino acids through macropinocytosis and lysosomal breakdown of extracellular proteins, which is exploited by cancer cells
to grow in nutrient-poor tumors. Here, through genetic screens in defined nutrient conditions we characterized LYSET, a transmembrane protein (TMEM251) selectively required when cells consume extracellular proteins. LYSET was found to associate in the Golgi with GlcNAc-1-phosphotransferase, which targets catabolic enzymes to lysosomes through mannose-6-phosphate modification. Without LYSET, GlcNAc-1-phosphotransferase was unstable owing to a hydrophilic transmembrane domain. Consequently, LYSET-deficient cells were depleted of lysosomal enzymes and impaired in turnover of macropinocytic and autophagic cargoes. Thus, LYSET represents a core component of the lysosomal enzyme trafficking pathway, underlies the pathomechanism for hereditary lysosomal storage disorders, and may represent a target to suppress metabolic adaptations in cancer.

○ Rationale: Discovery of a new co-factor important for GlcNAc-1-phosphotransferase and thus for Mannose-6-Phosphate generation and lysosomal trafficking

10. Mechanism-based heparanase inhibitors reduce cancer metastasis in vivo

○ Casper de Boer 1, Zachary Armstrong 2 3, Vincent A J Lit 1, Uri Barash 4, Gijs Ruijgrok 1, Ilanit Boyango 4, Merle M Weitzenberg 1, Sybrin P Schröder 1, Alexi J C Sarris 1, Nico J Meeuwenoord 1, Pedro Bule 2 5, Yasmine Kayal 4, Neta Ilan 4, Jeroen D C Codée 1, Israel Vlodavsky 4, Herman S Overkleeft 1, Gideon J Davies 2, Liang Wu 2 6

○ Proc Natl Acad Sci U S A

○ https://pubmed.ncbi.nlm.nih.gov/35881786/

○ Heparan sulfate proteoglycans (HSPGs) mediate essential interactions throughout the extracellular matrix (ECM), providing signals that regulate cellular growth and development. Altered HSPG composition during tumorigenesis strongly aids cancer progression. Heparanase (HPSE) is the principal enzyme responsible for extracellular heparan sulfate catabolism and is markedly up-regulated in aggressive cancers. HPSE overactivity degrades HSPGs within the ECM, facilitating metastatic dissemination and releasing mitogens that drive cellular proliferation. Reducing extracellular HPSE activity reduces cancer growth, but few effective inhibitors are known, and none are clinically approved. Inspired by the natural glycosidase inhibitor cyclophellitol, we developed nanomolar mechanism-based, irreversible HPSE inhibitors that are effective within physiological environments. Application of cyclophellitol-derived HPSE inhibitors reduces cancer aggression in cellulo and significantly ameliorates murine metastasis. Mechanism-based irreversible HPSE inhibition is an unexplored anticancer strategy. We demonstrate the feasibility of such compounds to control pathological HPSE-driven malignancies.
11. Sulfation of sialic acid is ubiquitous and essential for vertebrate development

- Rationale: Specific activity inhibitor of heparanase is a novel and a way of targeting tumor growth

- **Rationale:** Lots of folks studying sulfating pathways on our floor!
- **Rationale #2:** This paper is exciting as it highlights a less common sialic acid modification that is much more prevalent in nature than previously perceived. They identify two enzymes responsible for the synthesis of this modification and demonstrate that they could be essential for vertebrate development.

12. Glycan degradation promotes macroautophagy.

- **Rationale:** Glycosylation of proteins and lipids occurs in vertebrates, usually terminating with sialylation, which regulates the physicochemical and biological properties of these glycoconjugates. Although less commonly known, sialic acid residues also undergo various modifications, such as acetylation, methylation, and sulfation. However, except for acetylation, the enzymes or functions of the other modification processes are unknown. To the best of our knowledge, this study is the first to demonstrate the ubiquitous occurrence of sulfated sialic acids and two genes encoding the sialate: O-sulfotransferases 1 and 2 in vertebrates. These two enzymes showed about 50% amino acid sequence identity, and appeared to be complementary to each other in acceptor substrate preferences. Gene targeting experiments showed that the deficiency of these genes was lethal for medaka fish during young fry development and accompanied by different phenotypes. Thus, the sulfation of sialic acids is essential for the vertebrate development.

- **Rationale:** Macroautophagy promotes cellular homeostasis by delivering cytoplasmic constituents to lysosomes for degradation [Mizushima, *Nat. Cell Biol.* 20, 521-527 (2018)]. However, while most studies have focused on the mechanisms of protein degradation during this process, we report here that macroautophagy
also depends on glycan degradation via the glycosidase, α-l-fucosidase 1 (FUCA1), which removes fucose from glycans. We show that cells lacking FUCA1 accumulate lysosomal glycans, which is associated with impaired autophagic flux. Moreover, in a mouse model of fucosidosis—a disease characterized by inactivating mutations in FUCA1 [Stepien et al., Genes (Basel) 11, E1383 (2020)]-glycan and autophagosome/autolysosome accumulation accompanies tissue destruction. Mechanistically, using lectin capture and mass spectrometry, we identified several lysosomal enzymes with altered fucosylation in FUCA1-null cells. Moreover, we show that the activity of some of these enzymes in the absence of FUCA1 can no longer be induced upon autophagy stimulation, causing retardation of autophagic flux, which involves impaired autophagosome-lysosome fusion. These findings therefore show that dysregulated glycan degradation leads to defective autophagy, which is likely a contributing factor in the etiology of fucosidosis.

○ Rationale: Novel concept that fucose degradation is essential for macroautophagy
○ Rationale #2: The article presents novel fundamental knowledge about how glycan degradation is essential for successful macroautophagy. The authors highlight a specific glycosidase, FUCA1, which aids in fucose removal from glycans and thus, aids in cellular homeostasis maintenance.

13. Wild and domestic animals variably display Neu5Ac and Neu5Gc sialic acids
○ Nikoloz Nemanichvili 1, Cindy M Spruit 2, Alinda J Berends 1, Andrea Gröne 1, Jolianne M Rijks 3, Monique H Verheije 1, Robert P de Vries 2
○ Glycobiology
○ https://pubmed.ncbi.nlm.nih.gov/35648131/
○ Sialic acids are used as a receptor by several viruses and variations in the linkage type or C-5 modifications affect the binding properties. A species barrier for multiple viruses is present due to α2,3- or α2,6-linked sialic acids. The C-5 position of the sialic acid can be modified to form N-acetyleneuraminic acid (Neu5Ac) or N-glycolyneuraminic acid (Neu5Gc), which acts as a determinant for host susceptibility for pathogens such as influenza A virus, rotavirus, and transmissible gastroenteritis coronavirus. Neu5Gc is present in most mammals such as pigs and horses but is absent in humans, ferrets, and dogs. However, little is known about C-5 content in wildlife species or how many C-5 modified sialic acids are present on N-linked glycans or glycolipids. Using our previously developed tissue microarray system, we investigated how 2 different lectins specific for Neu5Gc can result in varying detection levels of Neu5Gc glycans. We used these lectins to map Neu5Gc content in wild Suidae, Cervidae, tigers, and European hedgehogs. We show that Neu5Gc content is highly variable among
different species. Furthermore, the removal of N-linked glycans reduces the binding of both Neu5Gc lectins while retention of glycolipids by omitting methanol treatment of tissues increases lectin binding. These findings highlight the importance of using multiple Neu5Gc lectins as the rich variety in which Neu5Gc is displayed can hardly be detected by a single lectin.

- **Rationale:** Growing use of probes to detect Neu5Gc. Could be an interesting discussion since we appear to lack a "one-and-done" standard. Cross reactivity?

14. **α-Gal present on both glycolipids and glycoproteins contributes to immune response in meat-allergic patients**

- Neera Chakrapani 1, Jörg Fischer 2, Kyra Swiontek 3, Française Codreanu-Morel 4, Farah Hannachi 4, Martine Morisset 4, Clément Mugemana 5, Dmitry Bulaev 6, Simon Blank 7, Carsten Bindslev-Jensen 8, Tilo Biedermann 9, Markus Ollert 10, Christiane Hilger 11

- **Background:** The α-Gal syndrome is associated with the presence of IgE directed to the carbohydrate galactose-α-1,3-galactose (α-Gal) and is characterized by a delayed allergic reaction occurring 2 to 6 hours after ingestion of mammalian meat. On the basis of their slow digestion and processing kinetics, α-Gal-carrying glycolipids have been proposed as the main trigger of the delayed reaction. **Objective:** We analyzed and compared the in vitro allergenicity of α-Gal-carrying glycoproteins and glycolipids from natural food sources. **Methods:** Proteins and lipids were extracted from pork kidney (PK), beef, and chicken. Glycolipids were purified from rabbit erythrocytes. The presence of α-Gal and IgE binding of α-Gal-allergic patient sera (n = 39) was assessed by thin-layer chromatography as well as by direct and inhibition enzyme-linked immunosorbent assay. The in vitro allergenicity of glycoproteins and glycolipids from different meat extracts was determined by basophil activation test. Glycoprotein stability was evaluated by simulated gastric and intestinal digestion assays. **Results:** α-Gal was detected on glycolipids of PK and beef. Patient IgE antibodies recognized α-Gal bound to glycoproteins and glycolipids, although binding to glycoproteins was more potent. Rabbit glycolipids were able to strongly activate patient basophils, whereas lipid extracts from PK and beef were also found to trigger basophil activation, but at a lower capacity compared to the respective protein extracts. Simulated gastric digestion assays of PK showed a high stability of α-Gal-carrying proteins in PK. **Conclusion:** Both α-Gal-carrying glycoproteins and glycolipids are able to strongly activate patient basophils. In PK and beef, α-Gal epitopes seem to be less abundant on glycolipids than on
glycoproteins, suggesting a major role of glycoproteins in delayed anaphylaxis upon consumption of these food sources.

- **Rationale:** This is becoming a more recognized problem as ticks/lyme disease spreads.

15. **Lysosomal cathepsin D mediates endogenous mucin glycodomain catabolism in mammals**

- Kayvon Pedram 1 2, Nouf N Laqtom 2 3, D Judy Shon 1 2, Alessandro Di Spiezio 4, Nicholas M Riley 1 2, Paul Saftig 4, Monther Abu-Remaileh 2 3, Carolyn R Bertozzi 1 2 5
- Proc Natl Acad Sci U S A
- https://pubmed.ncbi.nlm.nih.gov/36122205/
- Mucins are functionally implicated in a range of human pathologies, including cystic fibrosis, influenza, bacterial endocarditis, gut dysbiosis, and cancer. These observations have motivated the study of mucin biosynthesis as well as the development of strategies for inhibition of mucin glycosylation. Mammalian pathways for mucin catabolism, however, have remained underexplored. The canonical view, derived from analysis of N-glycoproteins in human lysosomal storage disorders, is that glycan degradation and proteolysis occur sequentially. Here, we challenge this view by providing genetic and biochemical evidence supporting mammalian proteolysis of heavily O-glycosylated mucin domains without prior deglycosylation. Using activity screening coupled with mass spectrometry, we ascribed mucin-degrading activity in murine liver to the lysosomal protease cathepsin D. Glycoproteomics of substrates digested with purified human liver lysosomal cathepsin D provided direct evidence for proteolysis within densely O-glycosylated domains. Finally, knockout of cathepsin D in a murine model of the human lysosomal storage disorder neuronal ceroid lipofuscinosis 10 resulted in accumulation of mucins in liver-resident macrophages. Our findings imply that mucin-degrading activity is a component of endogenous pathways for glycoprotein catabolism in mammalian tissues
- **Rationale:** Identification of cathepsin D as a novel mucin catabolic enzyme

16. **Establishment of fetomaternal tolerance through glycan-mediated B cell suppression.**

- G Rizzuto 1, J F Brooks 2, S T Tuomivaara 3 4 5, T I McIntyre 6, S Ma 7, D Rideaux 7, J Zikherman 2 6 8, S J Fisher 3 4 6, A Erlebacher 9 10 11 12
- Nature
- https://pubmed.ncbi.nlm.nih.gov/35236989/
- Discrimination of self from non-self is fundamental to a wide range of immunological processes. During pregnancy, the mother does not recognize the
placenta as immunologically foreign because antigens expressed by trophoblasts, the placental cells that interface with the maternal immune system, do not activate maternal T cells. Currently, these activation defects are thought to reflect suppression by regulatory T cells. By contrast, mechanisms of B cell tolerance to trophoblast antigens have not been identified. Here we provide evidence that glycan-mediated B cell suppression has a key role in establishing fetomaternal tolerance in mice. B cells specific for a model trophoblast antigen are strongly suppressed through CD22-LYN inhibitory signalling, which in turn implicates the sialylated glycans of the antigen as key suppressive determinants. Moreover, B cells mediate the MHC-class-II-restricted presentation of antigens to CD4+ T cells, which leads to T cell suppression, and trophoblast-derived sialoglycoproteins are released into the maternal circulation during pregnancy in mice and humans. How protein glycosylation promotes non-immunogenic placental self-recognition may have relevance to immune-mediated pregnancy complications and to tumour immune evasion. We also anticipate that our findings will bolster efforts to harness glycan biology to control antigen-specific immune responses in autoimmune disease.

- **Rationale:** Seems to describe the glycan-driving mechanism by which the fetus is not rejected by the mother, likely mediated by glycans produced by fetal tissue, which appear to bathe distant maternal sites and impact B-cell function -- FASCINATING!

**17. Using NMR to Dissect the Chemical Space and O-Sulfation Effects within the O- and S-Glycoside Analogues of Heparan Sulfate**

- Maria C Z Meneghetti 1, Lucy Naughton 2 3, Conor O'Shea 4 3, Dindet S-E Koffi Teki 5, Vincent Chagnault 5, Helena B Nader 1, Timothy R Rudd 6 7, Edwin A Yates 7, José Kovensky 5, Gavin J Miller 4 3, Marcelo A Lima 2 3
- ACS Omega
- [https://pubmed.ncbi.nlm.nih.gov/35874203/](https://pubmed.ncbi.nlm.nih.gov/35874203/)

Heparan sulfate (HS), a sulfated linear carbohydrate that decorates the cell surface and extracellular matrix, is ubiquitously distributed throughout the animal kingdom and represents a key regulator of biological processes and a largely untapped reservoir of potential therapeutic targets. The temporal and spatial variations in the HS structure underpin the concept of “heparanome” and a complex network of HS binding proteins. However, despite its widespread biological roles, the determination of direct structure-to-function correlations is impaired by HS chemical heterogeneity. Attempts to correlate substitution patterns (mostly at the level of sulfation) with a given biological activity have been made. Nonetheless, these do not generally consider higher-level conformational effects at the carbohydrate level. Here, the use of NMR chemical
shift analysis, NOEs, and spin-spin coupling constants sheds new light on how different sulfation patterns affect the polysaccharide backbone geometry. Furthermore, the substitution of native $O$-glycosidic linkages to hydrolytically more stable $S$-glycosidic forms leads to observable conformational changes in model saccharides, suggesting that alternative chemical spaces can be accessed and explored using such mimetics. Employing a series of systematically modified heparin oligosaccharides (as a proxy for HS) and chemically synthesized $O$- and $S$-glycoside analogues, the chemical space occupied by such compounds is explored and described.

- **Rationale:** While this paper is less biologically-focused than others, it demonstrates some of the capabilities of NMR to provide dynamic structural information about heparan sulfate that may be crucial for protein-HS interactions, but that is difficult to probe by other techniques.

### 18. The structure of EXTL3 helps to explain the different roles of bi-domain exostosins in heparan sulfate synthesis

- L F L Wilson, T Dendooen, S W Hardwick, A Echevarría-Poza, T Tryfona, K B R M Krogh, D Y Chirgadze, B F Luisi, D T Logan, K Mani, P Dupree

- Nat Commun
- [https://pubmed.ncbi.nlm.nih.gov/35676258/](https://pubmed.ncbi.nlm.nih.gov/35676258/)

Heparan sulfate is a highly modified O-linked glycan that performs diverse physiological roles in animal tissues. Though quickly modified, it is initially synthesised as a polysaccharide of alternating $\beta$-D-glucuronosyl and N-acetyl-$\alpha$-D-glucosaminyl residues by exostosins. These enzymes generally possess two glycosyltransferase domains (GT47 and GT64)-each thought to add one type of monosaccharide unit to the backbone. Although previous structures of murine exostosin-like 2 (EXTL2) provide insight into the GT64 domain, the rest of the bi-domain architecture is yet to be characterised; hence, how the two domains co-operate is unknown. Here, we report the structure of human exostosin-like 3 (EXTL3) in apo and UDP-bound forms. We explain the ineffectiveness of EXTL3's GT47 domain to transfer $\beta$-D-glucuronosyl units, and we observe that, in general, the bi-domain architecture would preclude a processive mechanism of backbone extension. We therefore propose that heparan sulfate backbone polymerisation occurs by a simple dissociative mechanism.

- **Rationale:** This article puts forth a novel/more detailed mechanism for heparan sulfate synthesis based on the structure of EXTL3. Heparan sulfate is an important glycan for many of our projects and this basic research study could prove a useful finding.
Lysosome-targeting chimeras (LYTACs) offer an opportunity for the degradation of extracellular and membrane-associated proteins of interest. Here, we report an efficient chemoenzymatic method that enables a single-step and site-specific conjugation of high-affinity mannose-6-phosphate (M6P) glycan ligands to antibodies without the need of protein engineering and conventional click reactions that would introduce "unnatural" moieties, yielding homogeneous antibody-M6P glycan conjugates for targeted degradation of membrane-associated proteins. Using trastuzumab and cetuximab as model antibodies, we showed that the wild-type endoglycosidase S (Endo-S) could efficiently perform the antibody deglycosylation and simultaneous transfer of an M6P-glycan from a synthetic M6P-glycan oxazoline to the deglycosylated antibody in a one-pot manner, giving structurally well-defined antibody-M6P glycan conjugates. A two-step procedure, using wild-type Endo-S2 for deglycosylation followed by transglycosylation with an Endo-S2 mutant (D184M), was also efficient to provide M6P glycan-antibody conjugates. The chemoenzymatic approach was highly specific for Fc glycan remodeling when both Fc and Fab domains were glycosylated, as exemplified by the selective Fc-glycan remodeling of cetuximab. SPR binding analysis indicated that the M6P conjugates possessed a nanomolar range of binding affinities for the cation-independent mannose-6-phosphate receptor (CI-MPR). Preliminary cell-based assays showed that the M6P-trastuzumab and M6P-cetuximab conjugates were able to selectively degrade the membrane-associated HER2 and EGFR, respectively. This modular glycan-remodeling strategy is expected to find wide applications for antibody-based lysosome-targeted degradation of extracellular and membrane proteins.

**Rationale:** Using glycan remodeling on antibodies to introduce Man-6-P glycans which will carry a protein of interest (bound by the antibody) into the lysosome via CI-MPR resulting in highly selective protein degradation. This technique (LYTAC) offers a mechanism to selectively degrade proteins which drive disease progression.