A Decade of Glycobiology: From R&D Systems to Bio-Techne

INTRODUCTION

About us

Bio-Techne can trace its roots to R&D Systems, which was incorporated in Minneapolis, Minnesota in 1976. Early products included hematology controls and calibrators for use in hospitals and clinics. Within a decade, R&D Systems was the first company to commercially produce the cytokine Transforming Growth Factor beta 1 (TGF- β 1). Over the years, R&D Systems and its sister brands have produced an everincreasing array of cell biology research products including proteins, antibodies, assays, cell culture reagents, and equipment. Meanwhile, in addition to developing the highest quality products for the market, Bio-Techne's scientists have consistently published peer reviewed research independently and in collaboration with academic investigators. As of this writing, we have authored or co-authored 65 papers since 1995, an average of nearly 3 publications a year.

Research in Glycobiology

Our entree into glycobiology was championed by Dr. Zhengliang "Leon" Wu. Leon joined the company after a stint as a glyco-scientist at MIT, where he conducted research on polysaccharide structure and function. In addition to product development, Leon and his team scientists began to publish peer-reviewed research. The primary goal of this endeavor was to make glycobiology more accessible to nonspecialists. Although most of our publications describe novel glycan assays for biologists, Bio-Techne scientists have also collaborated with academic investigators and advanced our understanding of the relationship between glycosylation and various biological events. What follows is a review of Bio-Techne's independently authored glycobiology assay development publications over the last decade.

You will see that our team has consistently worked at the leading edge of glycobiology research and product development. Thus, the objective of this research and development enterprise is to expedite our understanding of the central role of glycosylation in biology.



Dr. Zhengliang "Leon " Wu

biotechne

CUTTING EDGE ASSAYS DESIGNED TO EXPEDITE GLYCOBIOLOGY RESEARCH

For nearly a decade, our scientists have focused on developing user-friendly assays that expedite the investigation of the role of glycosylation in a broad range of experimental contexts.

A Versatile Polyacrylamide Gel Electrophoresis-Based Sulfotransferase Assay

Sulfation occurs via the activity of sulfotransferases, which transfer sulfates from donors to substrates. This post-translational modification (PTM) is important for the activity of hormones, growth factors, cytokines, viruses and bacteria among others. As of 2010, chromatography-based assays such as High-Performance Liquid Chromatography (HPLC), HPLC coupled Mass Spectrometry (MS) and Thin-Layer Chromatography (TLC) were the standard tools for analysis. However, these assays were not optimal for several reasons including low throughput and semiquantitative data output. Our first glycobiology paper, Wu *et al.*, 2010, described a versatile sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-based sulfotransferase assay. This novel assay (**Figure 1**) utilized sulfotransferases to catalyze the transfer of radiolabeled sulfate (³⁵S) from the donor substrate 3'-phosphoadenosine-5'-phosphosulfate (PAP³⁵S) to an acceptor substrate that is recognized by the sulfotransferase.



Reaction spiked with PAP³⁵S

Obtain radiogram of the dried gel

Identify and cut out hot spots on the gel

FIGURE 1. The reaction containing the enzyme and acceptor substrate was spiked with radioactive donor substrate and incubated for 20 minutes prior to being resolved on an 8% SDS gel. Radiolabeled product is subsequently counted using a scintillation counter.

At the time of publication, this novel assay had several advantages over existing methodologies. It allowed for increased throughput, as part of a drug screening process. It also allowed for the separation of mono-sulfated and di-sulfated small molecule products. Finally, this assay had the advantage of being broadly applicable to many substrates.

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View our Sulfotransferase Antibodies View our Sulfotransferases View our Sulfotransferase Activity Assays

A Universal, Phosphatase-Coupled Glycosyltransferase Assay

In 2011, we published a paper (Wu *et al.*, 2011) describing a novel assay that measured glycosyltransferase activity. Glycosyltransferases transfer glycans to specific acceptor sites on proteins or other glycans. Although investigators appreciated the importance of glycosylation for protein function, assaying this PTM remained a major challenge at the time. To address this challenge, we developed a novel assay that utilized coupling phosphatases to release inorganic phosphate subsequent to the activity of glycosyltransferases (**Figure 2**). This quantitative assay used malachite green reagents to detect free phosphate.



We found that this assay was universal to all glycosyltransferases that generate leaving groups with removable phosphates. Furthermore, this assay was quantitative so long as a phosphate was removed in a quantitative manner by the phosphatase. Finally, this nonradioactive assay could be performed in a 96-well plate format and did not require a separation step. In summary, this assay broke ground as a high throughput glycosyltransferase assay. Since this publication, this assay has become the mainstream method for studying glycosyltransferases and the paper has generated 70 citations.

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View Glycosyltransferase Activity Assays View Glycosyltransferases View Glycosyltransferase Antibodies

Detection of Specific Glycosaminoglycans and Glycan Epitopes by In Vitro Sulfation Using Recombinant Sulfotransferases

Also in 2011, our scientists described a novel assay designed to detect minute quantities of sulfated glycosaminoglycans (GAGs) in cells and tissues (Wu et., al., 2011). GAGs are commonly sulfated, linear polysaccharides that are part of the microenvironment that is critical for cellular differentiation, growth, migration, and survival. This paper was inspired by the fact that, at the time, GAG detection was difficult. The importance of GAG detection was further underscored by reports of adverse side effects and deaths caused by GAG contamination of Heparin (H), 3 years prior. In this assay, our scientists utilized sulfotransferases to detect GAGs and visualize glycan epitopes in tissue sections. Sulfotransferases catalyze the transfer of radiolabeled sulfate from the donor substrate PAPS³⁵S to the acceptor substrate. Using this assay, we found that commercially available Chondroitin Sulfate (CS) was contaminated with Heparan Sulfate (HS). Similarly, HS was found to have CS contamination. We also tested the commercially available food supplement Glucosamine Chondroitin and found that it too was contaminated with H and/or HS and probably Keratin Sulfate (KS). This approach has several advantages over metabolic labeling. It is extremely sensitive for GAG detection and suitable for detecting minute amount of GAG contaminants in pharmaceutical heparin preparation. Furthermore, pretreatment was not required, unless desired as part of experimental design. Finally, since this assay is applicable to any substrate that can be sulfated by sulfotransferases, investigators can make inferences about the acceptor substrate.

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Golgi-Resident PAP-specific 3'-phosphatase-coupled Sulfotransferase Assays

The next paper (Prather et al., 2011) continued our efforts to design sensitive and specific assays to assess sulfation. We previously described an assay that uses SDS-PAGE to quantitatively assess sulfation. Here we extended this principle to a nonradioactive high-throughput 96 well format. This was of particular interest to those involved in drug development. As described earlier, sulfotransferases transfer sulfate to the acceptor substrate, converting PAPS to PAP in the process. In this assay, Golgiresident PAP-specific 3 ' phosphatase (gPAPP) subsequently removed the 3 '-phosphate from PAP. By degrading PAP as part of this assay, one removes its inhibitory effect on the sulfotransferase reaction. The free inorganic phosphate was detected by malachite green reagents. This updated assay is comparable to the previously described radioisotope assay in sensitivity but is much more high-throughput compatible and much more user friendly. Finally, it is well suited for pharmacokinetic drug metabolism studies.



Detecting O-GIcNAc Using In Vitro Sulfation

O-linked β-N-acetylglucosamine, also known as O-GlcNAc is typically attached to serine or threonine via the process of O-GlcNAcylation. This glycan is important for many cellular functions including transcription, translation, and cell division. It also has been shown to be involved in maladies such as Alzheimer's disease, diabetes, and cancer. O-GlcNAc Transferase (OGT) attaches the O-GlcNAc while O-GlcNAcase (OGA) removes this glycan. It is unlike other glycans for several reasons¹. First, it has a relatively rapid on/off rate, similar to what is observed in other PTMs such as phosphorylation. Second, it is typically not extended via the attachment of other glycans. As with other glycans in previous publications, the work described in this report was designed to create an assay that reliably detects O-GlcNAc. This study (Wu et., al 2014) took advantage of the discovery that GlcNAc-specific Sulfotransferases can sulfate O-GlcNAc. In this paper, GlcNAc-specific sulfotransferases transferred radiolabeled sulfate from the donor substrate, PAP³⁵S to the acceptor substrate O-GlcNAc. This novel protocol was tested in peptides, recombinant proteins, and cell extracts. Sulfated GlcNAc was detected in the microgram range in cellular extracts and in the nanogram range on pure proteins or peptides. Finally, as with previous assays developed by our scientists, this assay was convenient, sensitive, and specific.

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Glycoprotein Labeling with Click Chemistry (GLCC) and Carbohydrate Detection

This publication (Wu et al., 2015) marked a major advance in Bio-Techne's effort to develop convenient, sensitive, and specific assays to detect glycosylation as well as to label glycoproteins. By the time this paper was published, there were several methods for localizing and visualizing glycans. Despite these advances, the utility of these approaches was constrained by the limited number of acceptor sites on target proteins. This paper introduced a novel method of glycan labeling called Glycoprotein/Protein Labeling with Click Chemistry (GLCC). Although click chemistry has been successfully applied to glycan labeling via metabolic methods, GLCC takes advantage of the specific activity of glycosyltransferases on acceptor substrates. In GLCC, glycosyltransferases attach clickable (azido) glycans to specific target acceptor sites. Click chemistry was then used to attach biotin to the azido glycan. Azido glycans were subsequently visualized with streptavidin-Horseradish Peroxidase (HRP) using standard SDS-Page methods. In summary, we found several advantages to GLCC. This method is far more specific and sensitive for glycan detection than any traditional method as the specificity is determined by labeling glycosyltransferases. The label is attached via covalent bonding, rendering the label stable and convenient for storage. Finally, as shown in subsequent publications, this method is adaptable for use with both chemiluminescence and fluorescence visualization technologies.

RESOURCES	
Visualize Terminal Glyc	ans

Non-radioactive Glycosyltransferase and Sulfotransferase Assay to Study Glycosaminoglycan Biosynthesis

This book chapter authored by Ethen *et al.*, 2015 detailed user-friendly protocols for nonradioactive colorimetric assays that can be used to understand the kinetics of Glycosaminoglycan (GAG) synthetic enzymes. The general principle of this assay has been described previously. Briefly, the glycosyltransferase or sulfotransferase transfers a glycan or sulfate from the donor substrate PAPS to the acceptor substrate that it is selective for. The donor substrate, PAPS, is converted to PAP and an inorganic phosphate is released from PAP by a coupling phosphatase. Finally, the inorganic phosphate is quantitatively detected using malachite green reagents.



Probing Sialoglycans on Fetal Bovine Fetuin with Azido Sugars Using Glycosyltransferase

This study (Wu *et al.*, 2016) used GLCC to probe sialoglycans on fetal bovine fetuin. Sialic acid is a negatively charged glycan that is typically found at the non-reducing ends of glycans. Sialoglycans have many important roles, including acting as receptors or ligands, and regulating the solubility, stability, and half life of secreted proteins. We were able to show that glycosyltransferases and glycosidases could be used in combination to map glycans on glycoproteins. **Figure 3** shows that the sialyltransferase ST3GAL1 only sialylates desialated fetuin, indicating that all of the sialic acid sites on fetuin are occupied prior to desialylation. This method has several advantages over other glycan labeling methodologies such as metabolic labeling. This method is specific, as azido-glycan specificity is determined by the glycosyltransferase or sialyltransferase used. Finally, this method can also be adapted to detect other types of glycans on glycoproteins.





Protein Staining

Streptavidin-HRP detection

FIGURE 3. Probing Sialoglycans using SDS-PAGE. Figure 3A gel shows total protein staining. Bovine Fetuin (left side) and desialated fetuin (right side) were incubated with increasing amounts of recombinant enzyme Human ST3GAL1 (5, 25, 50, 250 ng) and 0.5 nmol of the donor substrate CMP-Azido-Sialic Acid. The reactions were conjugated with Biotinylated Alkyne, sepatated by SDS Page, transferred to nitrocellulose and detected with Streptavidin-HRP via chemiluminescence. Incororation of Azido Sialic Acid the only detected on desialated fetuin (right side of Figure 3B) indicating that ST3GAL1 sialylates fetuin only after is desialylated

Non-reducing End Labeling of Heparan Sulfate Via Click Chemistry and High Throughput ELISA Assay for Heparanase

In this publication (Wu et al., 2017) we described a novel, high throughput enzyme-linked immunosorbent assay (ELISA) format Heparanase (HPSE) assay. HPSE is an endo- β -D-glucuronidase that hydrolyzes Heparan Sulfate (HS) at its sulfation domains. HPSE alters the integrity of the extracellular matrix by cleaving HS. Since HPSE activity has been linked to angiogenic responses, cell invasion, and cancer metastasis, it represents a potential anti-cancer target. Thus, high throughput HPSE activity assays are required for drug screening. In this ELISA, the substrate was generated as follows. The non-reducing ends of HS chains on recombinant human syndecan 4 (rhSydn4) were labeled with azido-GlcNAc (GlcNAz) using the HS specific glycosyltransferase heterodimeric exotosin 1 and 2 (EXT1/2). Click chemistry was used to biotinylate GlcNAz. The labeled rhSynd4 was immobilized to anti-Synd4 coated 96 well plates. Enzyme activity was visualized using streptavidin HRP which binds biotin. Increased HSPE activity was correlated with decreased absorbance at 450 nm on an ELISA plate reader. Compared to traditional assays, this method increased sensitivity at least by 1000-fold. Finally, since this highly sensitive (0.01 ng range) assay used non-reducing end labeled HS as a substrate, interference caused by labeling on the enzymatic activity was avoided.



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Imaging Specific Cellular Glycan Structures Using Glycosyltransferases Via Click Chemistry

Accumulating evidence supports the idea that altered glycosylation represents a useful cancer biomarker. T antigen, also known as core-1 glycan (Galß1-2GalNAC-R) is a putative biomarker. Another putative cancer biomarker, Tn antigen, is an intermediate product in the T antigen synthesis pathway. Th antigen occurs in the absence of the β -3 galactosyltransferase (C1GalT1) activity. Given the role of T/Tn antigen as a cancer biomarker as well as the lack of reliable methods for its detection, there is an imperative to develop methods to detect this antigen. To address this issue we applied our novel technology to the imaging of glycans in cultured cells (Wu et al., 2018a). Armed with our arsenal of azido-glycans, we visualized glycans in C3H10T1/2 mesenchymal stem cells and human umbilical vein endothelial cells (HUVECs). Glycosyltransferases were used to attach azido glycans to specific acceptor substrates in paraformaldehyde fixed cultured cells. Next, a biotin moiety was incorporated via click chemistry as described in previous publications. Finally, streptavidin-Alexa Fluor was used to visualize glycans in situ. Our findings indicated that we were able to visualize HS and T/Tn antigen in cultured cells. Glycoenzyme mediated visualization has the advantage of being more sensitive and more selective than metabolic assays, glycan binding proteins, and antibodies. This publication represents the first report on specific glycan imaging using glycosyltransferase and click chemistry. The publication was widely viewed among the scientific community and has been highlighted on the cover page of Glycobiology since 2019.





Fluorescence Visualize Terminal Glycans

Detecting and Imaging O-GlcNAc Sites Using Glycosyltransferases: A Systematic Approach to Study O-GlcNAc

In our most recent paper (Wu et al., 2018b), we were able to apply selective glycosyltransferase labeling to visualize open and closed O-GlcNAc sites in situ. As described in previous papers, O-GlcNAcylation is a reversible serine/threonine glycosylation that is involved in transcription, translation, and cell division. At a more granular level, the fine tuning of the ratio of open to closed sites is thought to be an important mediator of protein performance in the aforementioned contexts. Open O-GlcNAc sites are serine/threonine protein residues that lack O-GlcNAc. Conversely, closed sites are O-GlcNAcylated serine/threonine residues. In this paper we utilized recombinant glycosyltransferases to install and visualize azido-glycans on open and closed sites. Open sites were detected using OGT, which transfers GlcNAc to the serine or threonine residue. Closed sites were detected using β-1,3-N-acetylgalactosaminyltransferase (B3GALNT2), which attached azido-GalNAc (GalNAz) to O-GlcNAc. Use of OGT followed by B3GALNT2 allowed us to visualize the total number of O-GlcNAc sites. By using glycosyltransferases in this order, all sites are first closed and subsequently visualized using click chemistry and standard SDS-PAGE or fluorescence technology. In summary, we were able to demonstrate specific visualization of open and closed O-GlcNAc sites in biological samples using this approach.



Visualize Terminal Glycans Using Fluorescence

Visualize Terminal Glycans

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CONCLUSION

It is obvious that we have come a long way since the early radioactive assays. We have spent the last decade optimizing tools to improve the accessibility of glycobiology research to non-specialists. Our glycobiology research and development enterprise is ongoing and we will continue to publish our research and make novel technology available to glyco specialists and non-specialists alike. We hope you find this to be a useful resource and invite you to contact us if you have any questions or wish to collaborate.

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