High Throughput Screening of Glyco-biomarkers Using Plexera's Lectin Array and SPRi Platform, *PlexArray®*

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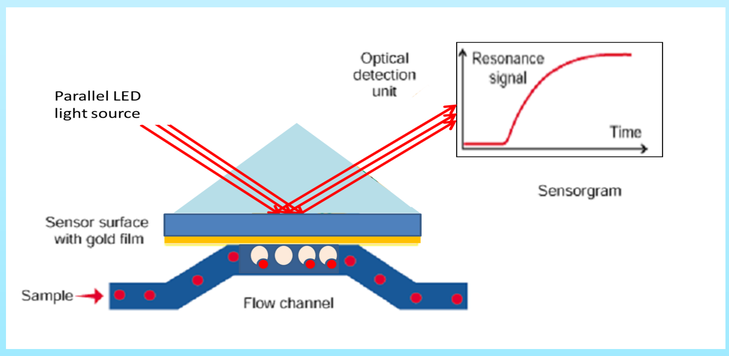
1. Introduction

Lectins are glycan-binding proteins that are important to many cellular functions such as cell adhesion, blood glycoprotein regulation, and innate immunity. The glycoform recognition function is essential in leading the immune system to remove pathogenic glycoproteins. Detailed studies of glycan structures are necessary to fully describe the glycome and the post-translational proteome and promise to play an major role in biomarker discovery. Glycosylation, the covalent attachment of carbohydrate groups (glycans), is the most common, yet structurally complex, of the post-translational modifications that occur naturally in proteins. Dysregulation of glycosylation is associated with a wide range of diseases including diabetes, cardiovascular disease, and cancer. For diagnosis, aberrant glycosylation has long been recognized as a hallmark of cancer with notable protein markers such as CA 19-9 consisting of glycan epitopes. More recently, specific cancer-induced glycoforms of classical serum proteins such as haptoglobin and transferrin have been observed and can potentially serve as markers for the physiological effects of the disease.

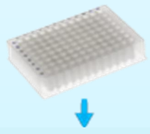
2. Lectin Array Principles

Lectins are glycan-binding proteins that serves many different biological functions and play a key role in cells and protein recognition pathways. Their ability to bind to soluble extracellular and intercellular glycoproteins allows them to be excellent glycoprotein screening agents. In combination with SPR microarray imaging technology, our product offers the highest throughput capacity for detecting glycan structures. Additionally, glycan affinities can be determined via the kinetic analysis features of our SPR system.

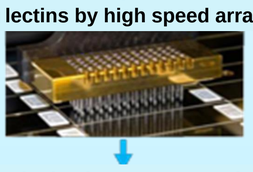


3. How it Works

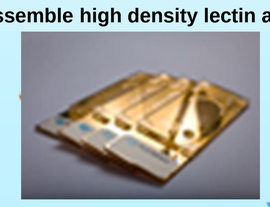
Preparation and loading of lectins into well plate



Printing lectins by high speed arrayer

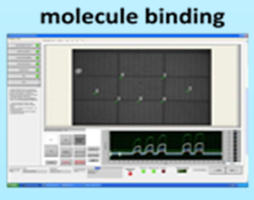


Assemble high density lectin array on SPRi gold chip surface

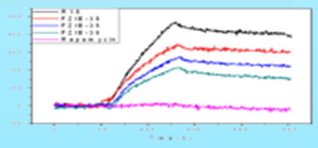


Each lectin with triplicate spots

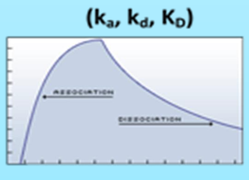
Real-time monitoring of molecule binding



Binding analysis of selected lectins to analytes

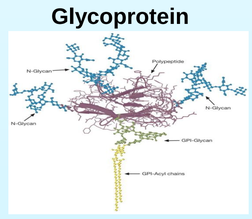


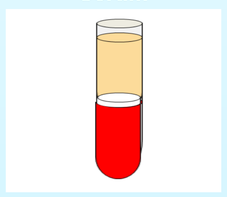
Dynamic binding (k_a, k_d, K_D)



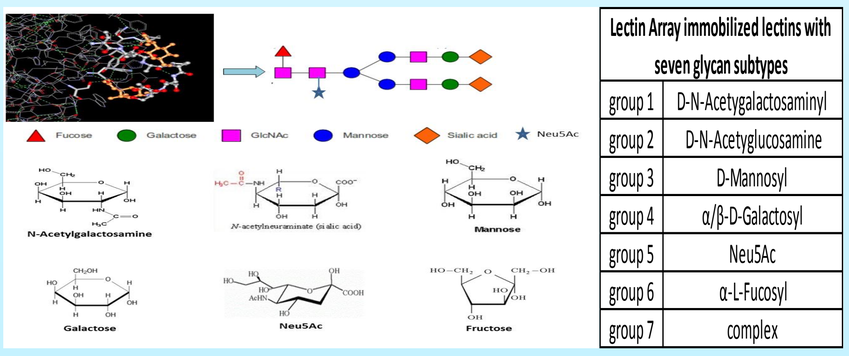
4. Glycoprotein & Lectin Array

Validated Glycoprotein | **Crude Cell lysate** | **Plasma Serum**



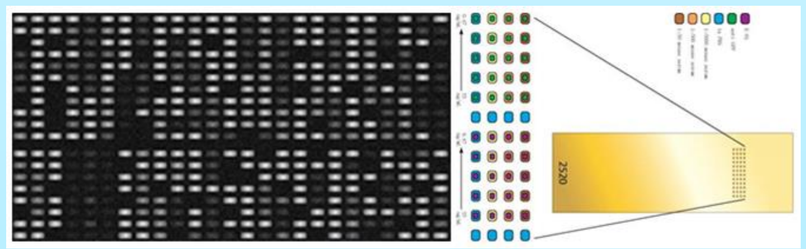


Glycan motifs

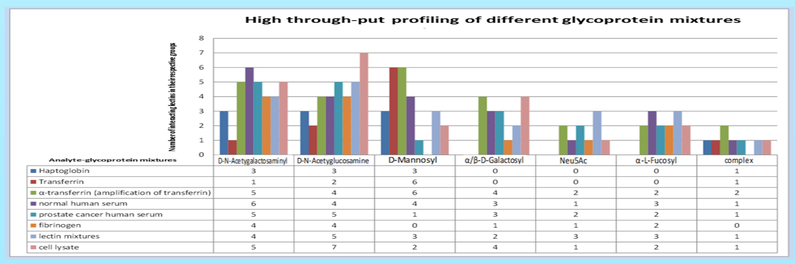


Lectin Array immobilized lectins with seven glycan subtypes	
group 1	D-N-Acetygalactosaminyl
group 2	D-N-Acetyglucosamine
group 3	D-Mannosyl
group 4	α/β-D-Galactosyl
group 5	Neu5Ac
group 6	α-L-Fucosyl
group 7	complex

Lectins arrayed on chip



Sugar motif analysis

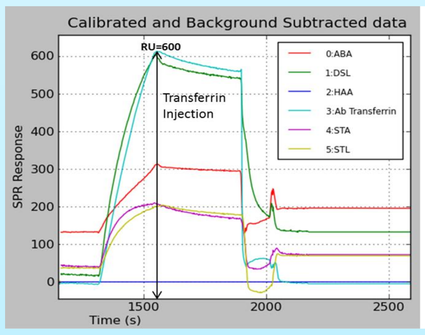


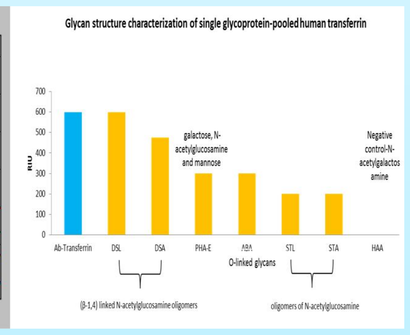
Samples from different resources can be easily analyzed with the lectin array and their corresponding sugar subtypes can be identified and characterized

5. Validation & Applications

5.1. Characterization of Single Glycoprotein

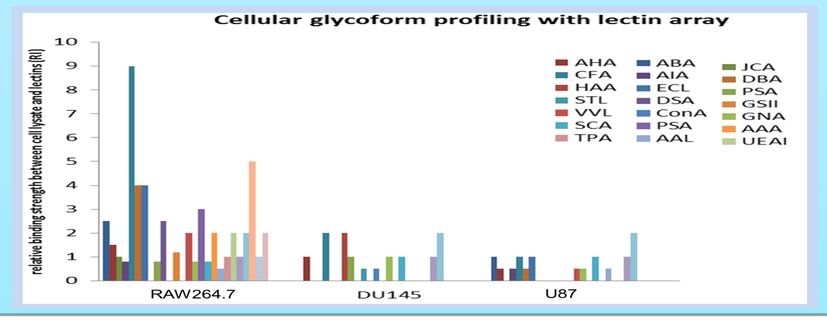
There are a variety of assays that can be performed using the Lectin Array Chip Kit. The most common and simple type is the purified individual glycosylated protein and lectin capturing experiment. Plexera's data analysis pipeline allows the calculation of kinetics on injected material and identification of glycan structures on the protein.





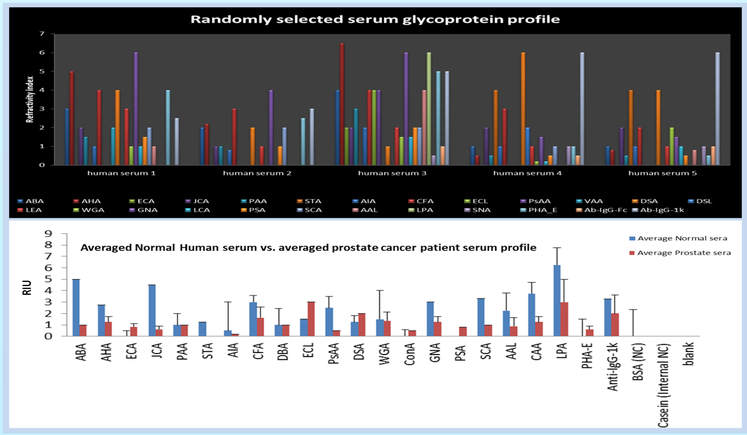
5.2. Cell Glycotypes

Complex protein mixtures such as cell lysates can be injected as well. This allows high-throughput profiling of cell membrane glycoprotein forms and intracellular glycoproteins. In the figure below, three different types of cell lysate were injected onto the Plexera lectin array and their bindings are summarized.



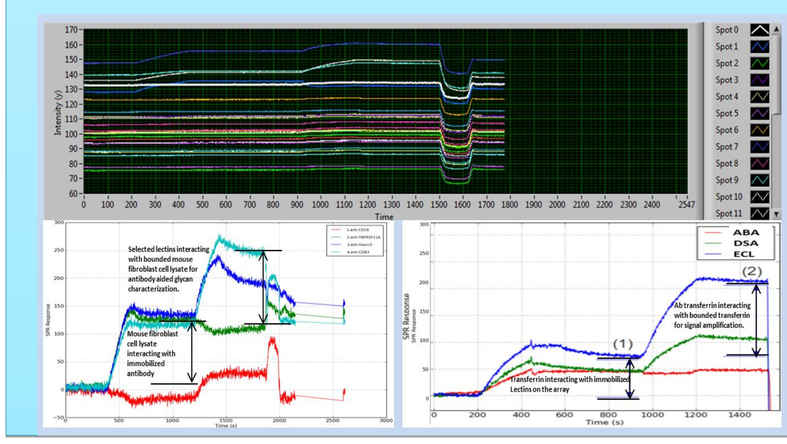
5.3. Serum Glyco-Profiling

The high-throughput comparison of serum glycoprotein can facilitate new field of glycoprotein biomarker discovery. Blood biomarkers are of great interest due to their accessibility. Aberrant glycosylation is a hallmark of cancer and circulating Advanced Glycation End products (AGEs) are associated with a number of chronic disease. Extensive study of these glycan structures can enhance the specificity and accuracy of discovered biomarkers. In the figures below, we demonstrated our lectin array's ability to profile sera and its capacity to detect potential glycan biomarkers.



5.4. Screening Low Abundancy Glycoforms

When injected analytes produce weak signals, secondary binding can be used to provide signal amplification. This can be done by injecting either an antibody over the captured lectin-analyte or lectin over captured antibody-analyte complex. The secondary antibody or lectin will boost the SPRi signal andverify the glycan identity; these complexes can later be eluted for additional analysis by mass spectrometry.



6. Conclusions

The lectin array and SPRi combination provides our customer advantages such as multiplexed interaction monitoring, simple operation, minimal sample usage, rapid data collection, and quantitative results. The label-free, real-time dynamic binding has been validated by both simple and complicated glycoprotein injections. Glycan-related information from crude cell lysates and serum have been successfully extracted from our lectin array platform. The SPRi-based chip is excellent for quickly screening of novel glycoform presented in complicated samples. Additional identification and characterization can then be performed via antibody array and MS-analysis. Most importantly, even low abundant glycoproteins can be detected through our additive SPR amplification. The array chip is guaranteed to remain functionally stable for six month in 4° C, making it a convenient yet powerful tool for high throughput glycoprotein biomarker discovery.

7. References

1. Angeloni, S., Ridet, J.L., Kusy, N., Gao, H., Crevoisier, F., Guinchard, S., et al. (2005). Glycoprofiling with micro-arrays of glycoconjugates and lectins. Glycobiology 15, 31–41.
2. Lausted C, et.al. Quantitative serum proteomics from surface plasmon resonance imaging. Mol Cell Proteomics. 2008;7(12):2464-74.
3. Images were selected from Geno and Giga