SPRi-based Lectin Array Chip

Glycoprotein screening application

*An efficient, label-free, high-throughput, real-time SPRi solution. The **Nanocapture® Lectin Chip** integrated screening technology is ideal for complex biological samples and glycol-biomarker discovery applications.*

FEATURES

- High throughput array format
- Label free technology
- Minimal sample preparation
- Rapid experimental process
- Quantitative kinetic result analysis
- Real time binding

BENEFITS

- Ideal solution for glycol-biomarker discovery
- Large scaled mixed glycoprotein screening process
- Quick identification of glycan structure
- Time and cost efficient
- Wide range of research applications
- Minimal procedural complexity

INTRODUCTION

Glycosylation is one of the most common post translational protein modifications. Dysregulation of glycosylation is associated with a wide range of diseases including diabetes, cardiovascular disease, and cancer. Although aberrant glycosylation has been recognized as a hallmark for cancer biomarker discovery, the complexity of the glycome has been discouraging comprehensive research in this field. Lectins are sugar-binding proteins that are highly specific for their respective glycoprotein conjugates (**Fig.1**). Their ability to bind to soluble glycoproteins allows them to be excellent glycoprotein screening agents. The Nanocapture Lectin Chip is a **S**urface **P**lasmon **R**esonance **i**maging (SPRi) technology developed for high-throughput glycoprotein biomarker discovery. The Nanocapture Lectin Chip offers label-free, rapid identification and monitoring of glycosylation changes in different types of samples.

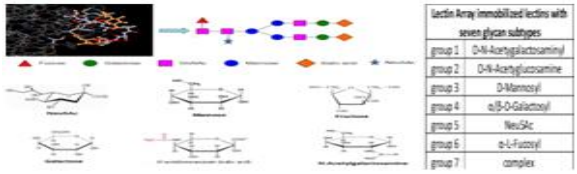

Fig.1. Typical human immunoglobin IgG with a fixed glycosylation site and diverse structural motifs.

ASSAY OVERVIEW

The ***PlexArray®*** HT System offers a powerful platform for the high-throughput quantitative measurement of molecular interactions in real time via SPRi technology. Utilizing glycan recognition pathway chemistry, a spectrum of protein mixtures can be flown over the array surface. The glycan and lectin interaction is then recorded in real-time graphical form which can elucidate the functional glyco-component in the mixture (**Fig.2**). Plexera's Lectin Chip and its unique analysis program, provides a label-free, high-throughput, highly efficient method to monitor and detect surface glycan structure.

PlexArray Platform-*Lectin-NanoChip™*
SPRi-based array

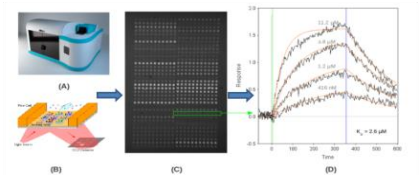

Fig.2. Lectin-glycoprotein interaction analysis with SPRi-based array

A. The Plexera SPRi system
B. SPRi flowcell containing a microarray
C. SPR image of a lectin array
D. SPR kinetic sensorgram from a lectin-glycoprotein experiment

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APPLICATION NOTE

PLEXERA®

Solutions for Functional Proteomics

LECTIN ARRAY CHIP

Plexera's Lectin Chip offers the largest throughput capacity for detecting glycan structure. On this SPR array chip, 41 different lectins from seven different sugar binding moieties: Acetygalactosamiyl, Mannosyl, Galactosyl, Fucosyl, Sialic Acid, Acetyglucosamine, and Neu5Ac, are immobilized on onto a single 1.4cm x 1.4cm region of a chip. Each Lectin Chip offers triplicate spots for each lectin for statistical reproducibility, resulting in a total of 270 simultaneously monitored interactions (**Fig. 3**). The Plexera lectin array chip also provides advantages such as easy operation, decreased sample usage, decreases experimental duration and de-convoluted quantitative results.

Table.1. Lectins immobilized on this chip

Spot position	Content on chip	Lectin name	Lectin Symbol	Source	MW(kDa)	Specificity/Suger
A1	System control					
A2	Lectin-1	Agaricus bisporus	ABA	mushroom	58.5	α/β-D-Galactosyl/Galactose
A3	Lectin-2	Arachis hypogaea	AHA	groundnut, peanut	120	α/β-D-Galactosyl
A4	Lectin-3	Erythrina cristagalli	ECA	cockspur coral-tree	56.8	α/β-D-Galactosyl
A6	Lectin-5	Phytolacca americana	PAA	pokeberry, pokeweed, scock	32	α/β-D-Galactosyl
A8	Lectin-7	Artocarpus integrifolia	AIA	Jack, Jack fruit	42	D-N-Acetygalactosaminyl
A9	Lectin-8	bovine serum albinium	BSA	bovine serum	114	D-N-Acetygalactosaminyl
A16	Lectin-15	Agglutinin, RCA120	RCA	castor oil palnt	120	D-N-Acetygalactosaminyl
A17	Lectin-16	Pisum sativum	VAA	mistletoe	49	D-Mannosyl
A18	Lectin-17	Datura stramonium	DSA	jimson weed, datura	86	D-N-Acetyglucosamine
A22	Lectin-21	Lycopersicon esculentum	LEA	Tomato	71	D-N-Acetyglucosamine
A32	Lectin-31	Anguilla anguilla	AAA	european freshweater eel	40	α-L-Fucosyl
A33	Lectin-32	Aleuria Aurantia	AAL	orange peel mushroom	72	α-L-Fucosyl
A34	Lectin-33	Tetragonolobus purpureas	TPA	asparagus pea	58-120	α-L-Fucosyl
A35	Lectin-34	Ulex europaeus agglutinin	UEA I	gorse, furze, whin	68	α-L-Fucosyl
A36	Lectin-35	Cicer arietinum	CAA	cerl bean, chick pea	44	Neu5Ac
A37	Lectin-36	Limulus polyphemus	LPA	horseshoe crab	400	α-L-Fucosyl
A38	Lectin-37	Maackia amurensis	MAA	amur maackia	130	Neu5Ac
A39	Lectin-38	Sambucus nigra	SNA	elderberry	140	Neu5Ac
A41	Lectin-40	Phaseolus vulgaris-L	PHA-L	(red kidney bean)	126	Complex

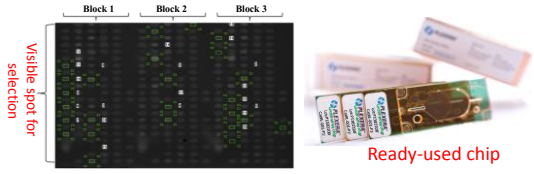

Fig. 3. Lectin Array SPRi chip containing 41 lectins and assay controls.

RESULTS & CONCLUSIONS

The Plexera Lectin array chip ensures detection specificity and sensitivity by both mechanical and software means. A uniquely designed surface and a controlled reaction chamber are used to prevent any environmental contribution to the fluctuation of the signal during screening. The Plexera analysis software removes spurious signals due to non-specific binding by subtracting signal from areas that surround the region of interest. Here we present a study using the lectin array chip to analyze the available glycoforms in a purified glycoprotein. (**Fig.4a**). The Lectin Chip was used to profile human serum from both healthy individuals and prostate cancer patients. Their lectin binding patterns and intensities are presented in the screened samples allows researchers to quickly identify disease-specific glycol biomarkers (**Fig.4b**).

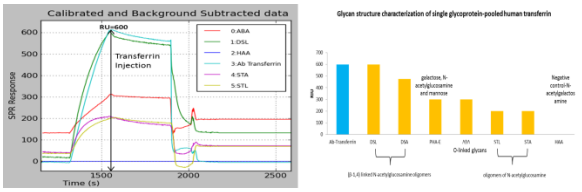

Fig. 4a. The interaction s of a single purified glycosylated protein with multiple lectins and its verifiend glycan characterization. Calibration and background subtraction is performed with Plexera's analysis software.

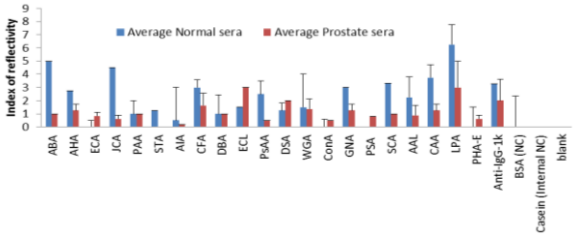

Fig. 4b.. High through-put screening of crude serum from 4 healthy individual and 4 prostate cancer individual. Easy SPRi operation and reusable high density PlexArray chip provide a powerful screening platform for new biomarker discoveries

Therefore, arraying lectins on a chip and exploiting the SPRi platform as demonstrated is a great way to characterize the glycosylation of proteins. It is a sensitive, high-throughput screening process, highly suitable for all phases of glycol-biomarker discovery. The Nanocapture Lectin Chip can be applied for:

- Glycoform Characterization
- Disease-relevant glycol-biomarkers
- Cell surface glycome profiling
- Pathogen detection
- Bacterial Tropism
- Cancer stem cell markers
- Altered glycan structure

REFERENCES

1. Lausted C, et.al. Quantitative serum proteomics from surface plasmon resonance imaging. Mol Cell Proteomics. 2008;7(12):2464-74.
2. Hirabayashi J,et.al. Glyco-catch method: A lectin affinity technique for glycoproteomics.
3. J Biomol Tech. 2002;13(4):205-18.

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