InnoScan scanner's Applications in Glycobiology

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Summary

Glycan arrays are becoming a standard tool in glycobiology. Here the major applications of glycan and lectin arrays using the InnoScan scanners are reviewed. Using microarray technologies, InnoScan scanner's users get the automation and high-throughput needed for glycomics analysis. Compared to standard microplate tools, microarray technologies have enormous advantages because of the miniaturization and multiplexing capabilities they provide, leading to saving of precious materials and reagents. Applications in cell biology, immunology and virology fields are described.

Glycan array basics

The biological importance of glycans in several cell functions is well known. The interaction between glycans and other biomolecules (especially glycan binding proteins, GBPs) has been proved to modulate several biological processes going from cell-matrix interactions to organ structure support to pathogen-host interaction during infection.

Glycobiology refers to the study of the structure and the function of glycans and their conjugates with proteins and lipids. One branch of glycobiology is the study of the interactions between glycans and other molecules (proteins, lipids, etc). Current methodologies for studying glycan: molecule interactions include plate-based assays such as ELISA tests in which a single glycan form is tested for interaction with glycan binding molecules at different concentrations. Despite the high specificity and sensitivity of plate-based assays, these technologies can only test for one glycan form at once. However, due to the complexity in the structure of glycan and glycoconjugates it is necessary to test several glycan forms, making the use of plate-based assays of great expense. High-throughput approaches allowing for testing several glycan forms in a single assay are then needed.

Current methodologies in array printing allow for the manufacturing of multiplexed assays in which several samples can be probed on the same slide. Glycan arrays are becoming a standard technique for screening glycan interactions with other molecules, especially glycan binding proteins (GBPs). In this way, the Glycomics Array Facility at the Institute for Glycomics of Griffith University uses printed microarray technology for high-throughput screening and resolution of glycan-binding profile of proteins. Using the InnoScan 1100 AL scanner, their process is fully automated for detection of the interaction between glycans and their corresponding GBPs (1).

A typical glycan array assay is schematized in **figure 1**: **A)** Glycans or lectins are

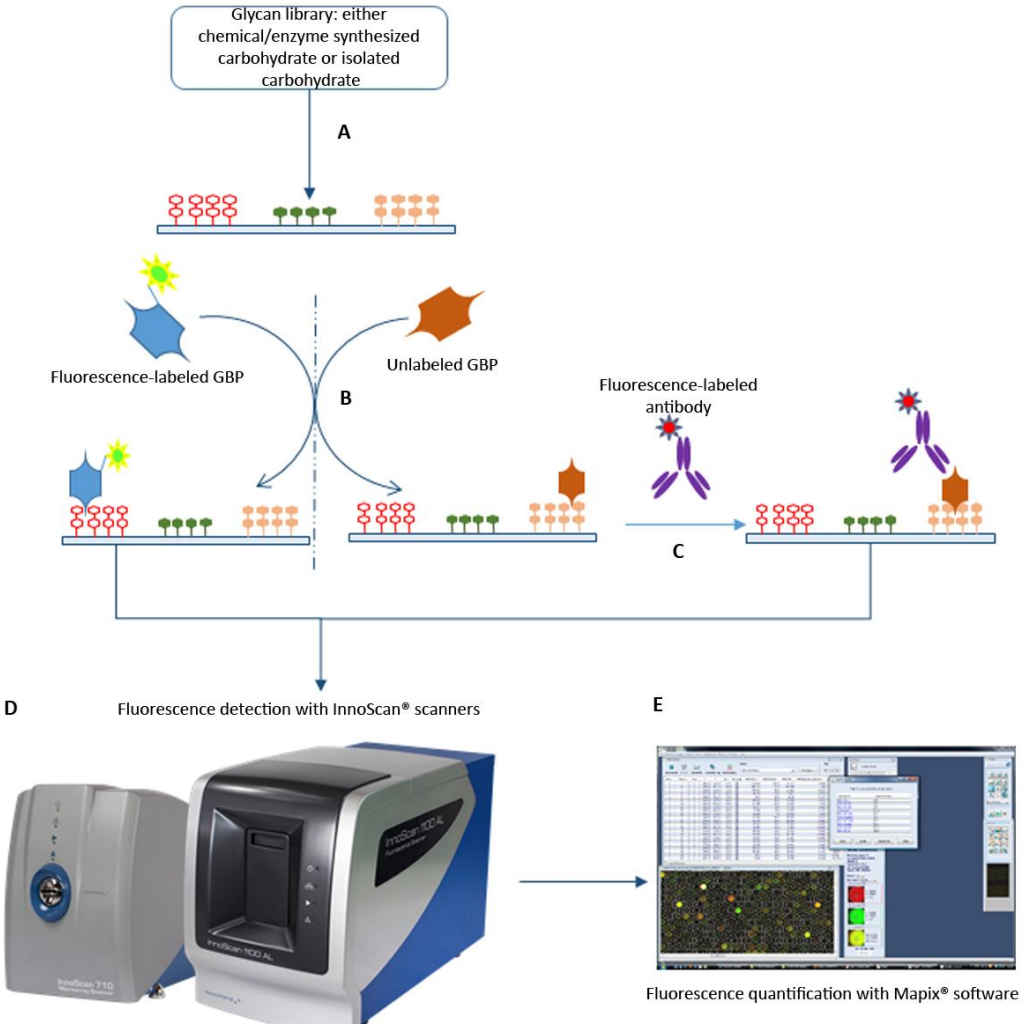

Figure 1. Glycan array workflow: Refer to the text for explanation

immobilized on functionalized glass slides using a microarray spotter. The immobilization occurs by either covalent binding or adsorption depending on the printed substrate. **B)** Glycan-binding molecules (either GBPs, virus, cells, or others) are put in contact with the spotted slide. The interaction between GBPs and the immobilized glycans is detected by fluorescence dyes, whereby the GBP is coupled to a fluorophore before the incubation step, or once the incubation step is done, a fluorescently-labeled antibody against the GBP is used in an additional step **(C)**. **D)** Microarray scanners are used to detect fluorescence. The Innopsys scanners are ideal tools to automate fluorescence detection with the ability to detect either two or three dyes simultaneously. **E)** Images are then analyzed to quantify fluorescence using the Mapix software, which in combination with the scanner assures a complete automation of fluorescence quantification.

Applications

Glycan arrays are becoming standard tools for the screening of GBPs for a very wide range of applications going from virology diagnosis to cancer research including vaccine development (Figure 2).

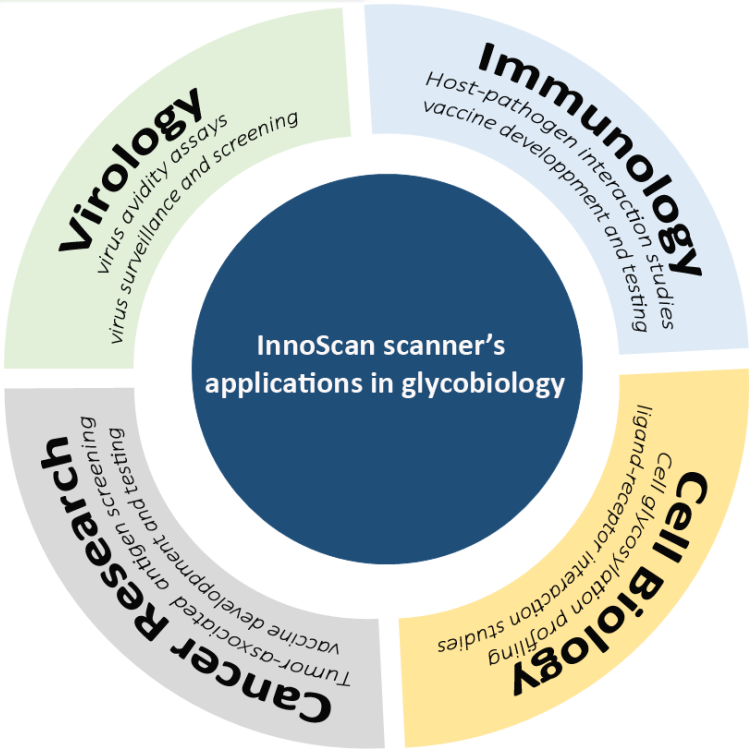

Figure 2: Major applications of glycan arrays done by Innopsys' users

Virus avidity assays

Glycan arrays can be used to characterize and monitor the influenza virus. McBride *et al* from the Scripps Research Institute have developed a 48-array slide to simultaneously detect the interaction of 6 different glycans with 6 GBPs at 8 dilutions in order to monitor the Influenza A virus hemagglutinin avidity specificity (2). The assay aims at monitoring if avian viruses are adapting to human-type receptors. The resulting assay gave results equivalent to standard-plate assays, but with a significant decrease in consumption of compounds and biologicals by 1,500x and 360x, respectively.

The Scripps Research Institute uses the InnoScan 1100AL for their glycan array assays. They use the simultaneous detection of three channels to detect three different kinds of spots on their slides: They use Atto488-NHS dye during the slide manufacturing process as grid marker/landing lights, then streptavidin-555 dye to look at biotinylated lectins with known specificity as controls; finally, they use an Alexa 647-labeled antibody to detect receptor-binding specificity of influenza A virus hemagglutinins.

Immunology research

Glycans have been shown as to act as key factors in modulating several mechanisms of the immunological response to pathogens. Glycan profiling of immune cells has shown differences in glycan structures between species. The group of Professor Paulson at the Scripps Research Institute has used lectin arrays to define the specificity of two antibodies from mouse and human, showing differences in CD22-related B cell maturation in each species. Even when the final outcome for the unmasking of CD22 in the germinal center was the same for both species, the mechanism by which this occurs is different in mouse and human B cells and is related to the glycan structure specificity of CD22 in

each species. Thanks to glycan arrays, the group of Pr. Paulson showed for the first time that human CD22 has preferences for sulfated glycans, whereas murine CD22 has preferences for the sialic acid Neu5Gc.

Cell biology research

Cell glycosylation profiling is necessary to better understand the role of carbohydrates in cell function and fate. Using lectin-based cell microarrays, the group of Dr. Katrlik from the Department of Glycobiology at the Slovak Academy of Science has defined the glycocalyx of equine native uncultured mural granulosa cells and *in vitro* cultured mural granulosa cells. Their innovative method consists in creating a lectin array on hydrogel slides on which different lectins are immobilized, then cells are cultured on the slides to follow the interaction between cell glycocalix and immobilized lectins.

Using the InnoScan 710 scanner, the group was able to study the affinity of cell glycocalyx with immobilized lectins at different concentrations, hence defining the glycosylation profile of the cells. The advantage of this innovative method is the capability of using live cells in their assays, diminishing the risk of glycoconjugate modification during glycan extraction. Results were validated by classical immunohistochemistry assays.

Conclusion

Carbohydrates are key effectors of numerous cell functions, as they have an essential role in the assembly of complex multicellular organs and organisms which requires interactions between cells and the surrounding matrix. Glycans and glycoconjugates are modulators of a wide variety of processes such as cell differentiation and immunity. Due to their structural complexity, the study of the glycome requires high-throughput techniques allowing for the testing of several glycan forms in a single experiment. Microarray technology applied to glycome analysis is becoming a standard methodology because it enables the simultaneous detection of hundreds of glycan forms on a single slide. Thanks to their miniaturization capabilities, glycan arrays allow for minimal consumption of precious materials and reagents, leading to reduction in experimental costs.

The InnoScan scanners are optimal tools for glycan array fluorescence detection allowing for the simultaneous detection of two or three dyes. Equipped with a 24-slide autoloader, they provide with the automation and high-throughput capabilities needed for glycomics analyses.

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References

1) Glycomics Array Facility, Institute for Glycomics, Griffith University Website: https://www.griffith.edu.au/science-aviation/institute-glycomics/facilities/glycan-array
2) McBride R., *et al*. A Miniaturized Glycan Microarray Assay for Assessing Avidity and Specificity of Influenza A Virus Hemagglutinins. *J. Vis. Exp.* (111), e53847, doi:10.3791/53847 (2016)
3) Macauley M.S., *et al*. Unmasking of CD22Co-receptor on Germinal Center B-cells Occurs by Alternative Mechanisms in Mouse and Man. J. Biol. Chem. 290(50):30066-30077, doi: 10.1074/jbc.M115.691337 (2015)
4) Accogli G., *et al*. A lectin-based cell microarray approach to analyze the mammalian granulosa cell surface glycosylation profile. *Glycoconj J*. 33(5):717-24. doi: 10.1007/s10719-016-9666-2 (2016)