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The 10th Anniversary of Carbohydrate Microarrays (2002 – 2012)

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Carbohydrates, like nucleic acids and proteins, are essential biological molecules. Owing to their intrinsic physicochemical properties, carbohydrates are capable of generating structural diversity in a multitude of ways and are prominently displayed on the surfaces of cell membranes or on the exposed regions of macromolecules. In human and virtually all animal species, cell-display of specific complex carbohydrates is characteristically associated with the stages or steps of embryonic development, cell differentiation, and transformation of normal cells to abnormally differentiated tumor or cancer cells [1-4]. Many microbial organisms carry unique glycosylation systems and are able to produce specific sugar signatures [5-10]. Importantly, there are multiple carbohydrate-recognition systems in living species that read the biological information of complex carbohydrates. Notably, these include the lectin-like Glycan-Binding Proteins (GBPs) for the carbohydrate-mediated cell-cell communication and signaling (see a comprehensive summary of GBPs at http://www.functionalglycomics.org/) and a universe of anti-glycan antibodies produced by many animal species that play key roles in protecting a subject from microbial infections [11-13]. Thus, carbohydrates are uniquely suitable for storing biological signals in the forms which are identifiable by other biological systems.

The birth of modern carbohydrate microarrays in 2002 brought in new high-throughput tools to explore the biological information content in the glycome, i.e., the universe of carbohydrate moieties in living organisms. Four research articles about carbohydrate microarrays first appeared in the scientific literature in 2002 [14,15]. These include polysaccharide and glycoconjugate microarrays, reported by Denong Wang's group at Columbia University's Genome Center (now at SRI International, CA, USA) [16,17]; monosaccharide chips, by Milan Mrksich and coworkers at the University of Chicago [18]; arrays of natural and synthetic neoglycolipids, by Ten Feizi's group at Imperial College Faculty of Medicine, Harrow, U.K. [19]; and arrays of synthetic oligosaccharides in microtiter plates, by a Scripps Research Institute group led by Chi-Huey Wong (currently, president of Academia Sinica, Taiwan) [20].

In the past ten years, a number of experimental approaches have been developed to construct carbohydrate microarrays. A timely, specialized book, "Carbohydrate microarrays, Methods and Protocols (Humana Press)", was edited by Dr. Yann Chevolot of Université de Lyon in France and printed in 2012 [21]. Carbohydrate microarrays can be produced using different methods. These include technologies that directly utilize underivatized carbohydrates in microarray construction, technologies that require chemical modification of carbohydrates before microarray fabrications, methods of non-covalent immobilization of carbohydrates, and methods of covalent coupling of saccharides on array substrates. There are also technologies that are designed to display saccharides in defined orientations or specific cluster configurations in order to resemble the native configuration of functional carbohydrate ligands.

The use of underivatized saccharides for microarray construction has the advantage of preserving the native structures of the carbohydrate molecules. It requires, however, a ready-to-use microarray surface with appropriate surface chemistry that can be directly used to fabricate comprehensive carbohydrate microarrays with underivatized carbohydrates from a wide range of sources. Methods currently in use include non-covalent binding of underivatized carbohydrate antigens by passive adsorption on a chip, such as nitrocellulose-coated glassslides [16] or black polystyrene surfaces [22] and methods for covalently immobilizing underivatized carbohydrates on a slide surface by appropriate chemical linking techniques [23-29].

Carbohydrate microarrays can also be fabricated by using derivatized carbohydrates. Due to the small molecular size and hydrophilic nature, most oligosaccharides cannot be directly immobilized onto nitrocellulose or black polystyrene surfaces for microarray applications. However, an oligosaccharide probe can be modified with a tag or coupled to a larger carrier molecule for non-covalent immobilization. Methods include non-covalent immobilization of derivatized carbohydrates in microarrays [19,30] or in Enzyme-Linked Immunosorbent Assay (ELISA) microtiter-plates [20], and covalent immobilization of derivatized carbohydrates in microarrays. The latter includes, but are not limited to, the popular Consortium for Functional Glycomics (CFG) printed glycan arrays [31,32] and various technologies of notable technical features that were developed independently [18,33-40].

Affinity immobilization is another class of approaches for the derivatized carbohydrates. For example, biotin-derivatized carbohydrates can be immobilized on a streptavidin-coated substrate through the affinity interaction of the streptavidin–biotin pair to create carbohydrate microarrays. Biotin-derivatized carbohydrates include carbohydrate ligands that are biotinylated via a short aliphatic spacer or at the peptide part of glycopeptides [32,41,42]. DNA-Directed Immobilization (DDI) is another practical strategy for immobilization of oligonucleotide glycomimetic conjugates on a chip surface for the preparation of carbohydrate microarrays [37-40].

Despite technical differences among different platforms of carbohydrate microarrays, they are solid phase binding assays and share a number of common characteristics and technical advantages. For instance, they contain the capacity to display a large panel of carbohydrates in a limited chip space, they are high-throughput

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quantitative assays, and they make an effective use of carbohydrate substances that are often difficult or cost-inefficient to synthesize.

In a carbohydrate microarray, each carbohydrate is spotted in an amount that is drastically smaller than that required for a conventional molecular or immunological assay. This technical feature ensures a condition that the binding of a molecule in solution phase to an immobilized micro-spot of ligand in the microarray substrates has minimal reduction of the molar concentration of the molecule in solution [43]. Thus, microarray-based assays are intrinsically optimized for binding equilibrium to take place, which is the basis for this class of the hyper-sensitive binding assays [44]. Carbohydrate microarrays have higher detection sensitivity than most conventional carbohydrate analytic tools, such as carbohydrate-specific ELISA and the glycolipid-based "Eastern blot" assays that were developed in 1980s by a number of early researchers in this field [45,46]. Historically, this situation is very similar to the relationship between conventional blotting methods for nucleic acids or proteins, such as Southern, Northern, and Western blots, and the nucleic acid–based or protein/peptide–based microarrays.

Carbohydrate microarrays constructed by various methods may differ in their technical characteristics and suitability for a given practical application. Some platforms may be applied complementarily to solve a practical question. For examples, the method of nitrocellulose-based immobilization of carbohydrate-containing macromolecules, including polysaccharides, glycoproteins and glycolipids, is suitable for the high-throughput construction of carbohydrate antigen microarrays [16,47,48] to support the large-scale immunological characterization of carbohydrate antigens and anti-carbohydrate antibodies. However, the detection specificity of this carbohydrate microarray would be at the level of a carbohydrate antigen, not a glyco-epitope, if the native carbohydrate antigens were spotted. This is owing to the fact that many carbohydrate antigens display multiple antigenic determinants or glyco-epitopes [12,49,50]. Examining the finer details of the binding properties would require the use of microarrays of defined oligosaccharide sequences. Oligosaccharide array-based binding assays can be applied, in combination with saccharide competition assays, to decipher precise saccharide components of a specific antigenic determinant or glyco-epitope [19,25,28,31].

A number of carbohydrate microarray platforms have reached or are very close to the technical stage of the current nucleic acid–based or protein-based microarrays that are readily available for practical uses. Technical issues that require immediate attention may include, but are not limited to, optimization of existing technologies for array construction, quality control and technical standardization in both microarray production and application, and establishment of specialized bioinformatic tools to handle the massive amount of carbohydrate microarray data and to effectively extract diagnostic or research information from each microarray assay.

Exploring the repertoires of glyco-epitopes represents, however, a long-term goal of glycomics research. It was estimated that the human glycome contains 10,000 to 20,000 minimal epitopes for glycan-binding proteins [51]. In considering the repertoires of the "hybrid" structures that are generated by protein posttranslational modification, including both *N*- and *O*-glycosylation, the repertoires of carbohydrate-related antigenic structures can be much larger. Furthermore, the conformational diversity of carbohydrates and micro-heterogeneity of carbohydrate chains substantially increases the repertoire of carbohydrate-based antigenic determinants or glyco-epitopes [10]. Including carbohydrate structures of the microbial world, which are directly relevant to medicine, the sizes and diversity of the repertoires of glyco-epitopes are unpredictable.

Joint effort by academic and industrial sectors is highly recommended to facilitate the establishment of libraries of carbohydrate probes, as well as monoclonal antibodies, lectins, and other carbohydrate-binding proteins. Using specific immunological probes to characterize glyco-epitopes is equally important to the structural determination of glyco-epitopes. Similar effort has been successfully made for protein-based biomarkers. A notable example is the establishment of a large collection of monoclonal antibodies for Cell Differentiation antigens (CD antigens). Availability of specific probes for CD antigens, in combination with the state-of-the art technologies of flow cytometry (High-Dimensional Fluorescence-Activated Cell Sorting, or Hi-D FACS) [52], has revolutionized research in cellular biology and immunology and medical applications of CD antigens, especially in the clinical diagnosis of leukemia and other human diseases. Exploring the repertoires of carbohydrate-based biomarkers and targeting agents, with the aid of carbohydrate microarray technologies and other high-throughput Omics tools, may represent one of the highly active areas of post-genomics research in the coming years, which will likely be accompanied by a fruitful outcome in the glycomics-oriented biotech industry for diagnostics, therapeutics, and vaccines.

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