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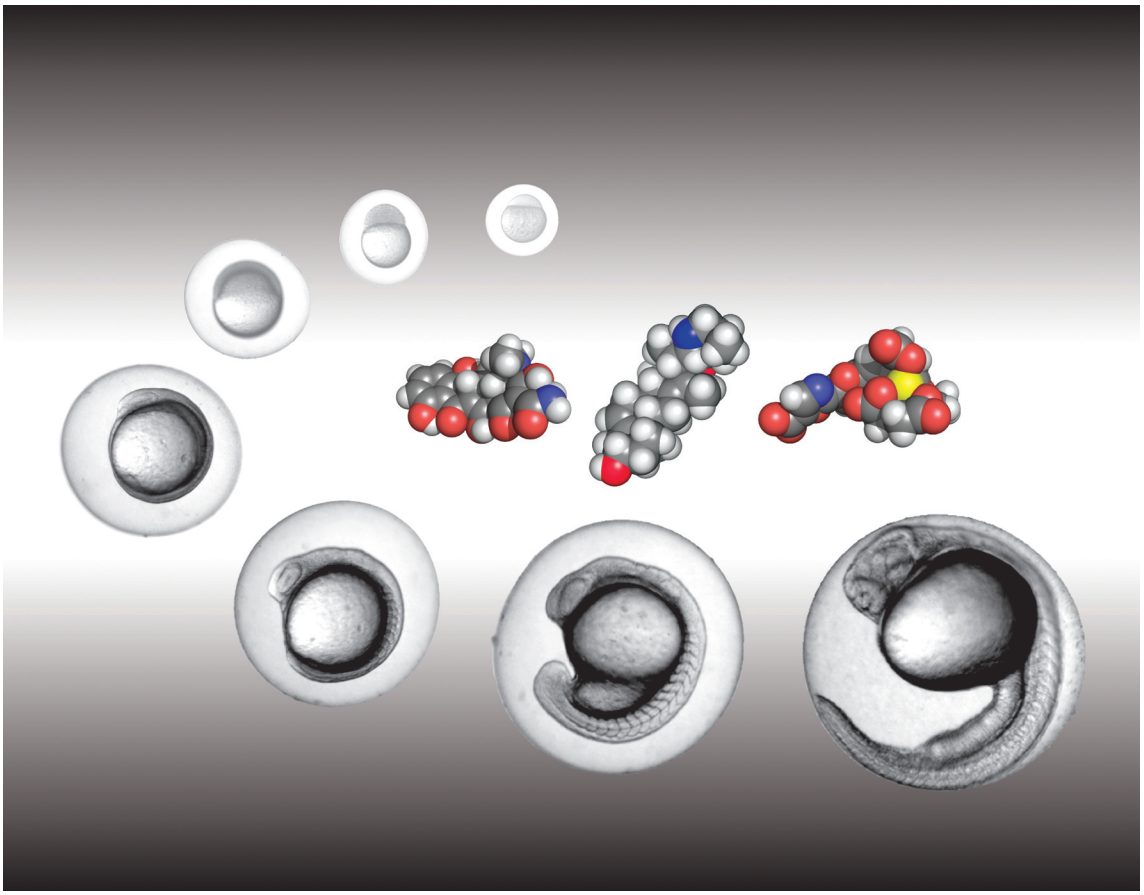

Carbohydrate arrays as tools for research and diagnostics†

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In a very short time, carbohydrate microarrays have become important tools to investigate binding events that involve sugars. High throughput analysis of carbohydrate interactions with a wide range of binding partners, including proteins, RNA, whole cells and viruses, can be performed. Questions ranging from simple binding events to in-depth kinetic analysis can be addressed. This *tutorial review* summarizes methods to produce carbohydrate microarrays as well as their use. Some selected examples illustrate applications and the potential that these tools hold.

1. Introduction

Sugars are an important part of our lives—we are literally covered with them. For a long time, biochemical research focused on dietary sugars and their metabolic pathways are commonly taught. In contrast, relatively little is known about oligo- and polysaccharide function in the organism despite their importance and ubiquitous presence.[1,2]

Carbohydrate complexity and the lack of research tools have complicated investigations into this class of biooligomers. In recent years, carbohydrate research has gained increased interest as the function of cells and organisms cannot be explained by proteins and nucleic acids alone. New tools have been developed to fuel carbohydrate research[3,4] and carbohydrate microarrays are particularly well suited to study interactions involving cell surface carbohydrates.

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2. Cellular oligo- and polysaccharides

All cells are surrounded by a layer that is largely made up of carbohydrates.[1,2] Most oligo- and polysaccharides are cell surface carbohydrates or part of the extracellular matrix, while only few polysaccharides are found inside cells. Cell surface sugars are either part of the protective layer that shields cells from harmful physical forces or regulate interactions of cells with the environment. Thus, carbohydrates are involved in most cell–cell interactions, cell motility and cell adhesion processes.

Oligo- and polysaccharides do not exist as free sugars, but are attached to proteins and lipids. The sugar chains are synthesized by glycosyltransferases,[5] trimmed by glycosylases, and often further modified. The glycome, the different carbohydrate structures present on cells and organisms, is determined by the cell type-, differentiation- or condition-dependent expression and activity of many sugar-modifying enzymes. The synthesis of carbohydrates is less well organized than that of nucleic acids and proteins. The enzymes do not act on all potential carbohydrate substrates to result in carbohydrate heterogeneity.

Cell surface sugars are involved in most processes that involve cell interactions with their environment, including

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differentiation, inflammation, fertilization, apoptosis and cell growth.[1] Carbohydrates act in a variety of ways to transmit signals. Some sugars are classical ligands or co-receptors that facilitate cell attachment or mediate signaling. Glycosaminoglycans of the mammalian cell surface serve as co-receptors for proteins that affect the respective cells. Other carbohydrates act indirectly by regulating interactions of molecules *via* steric hindrance, exclusion or recruitment. Thus, carbohydrates guide cell interactions by initiating or preventing attachment or motility.

Aberrant expression of carbohydrates is associated with many diseases including cancer and, thus, they are also important drugs and drug targets.[6,7] Most prominent is the anti-coagulant heparin.[8] Efforts are underway to exploit the cell specific expression of carbohydrates for cell targeting. Carbohydrates or carbohydrate binding proteins are also present on the cell surface of pathogens and can mediate their cellular uptake. Unique carbohydrate structures on pathogens are exploited to generate carbohydrate-based vaccines.[9,10] Some viruses and bacteria use cell surface sugars to gain entry into host cells.[11,12] Blocking carbohydrate–protein interactions with inhibitors such as Tamiflu at an early stage can terminate influenza virus infections.

3. Challenges of carbohydrate research

Carbohydrate complexity is a major challenge for studies focusing on interactions with other biomolecules.[3,13] The theoretical number of structures that can be assembled from a given number of sugar residues exceeds by far the number of combinations possible for the linear nucleic acids and peptides. The lack of a template driven synthesis may result in a tremendous number of different carbohydrate structures present on a single cell. The identification of specific carbohydrate sequences responsible for a particular function is an essential first step for glycomics investigations.[14,15] The purification of a polysaccharide is a *tour de force* since many closely related carbohydrates are present and similar sugars possess similar physical properties. Mass spectrometry has eased this process, but it is still not routine to determine the exact structure of complex polysaccharides.

The assessment of the biological function of a particular carbohydrate remains challenging. Knock-out techniques yield insights into sugar function and interactions,[16] but are time-consuming. In addition, knocking-out a glycosyltransferase disturbs the synthesis of many carbohydrates, thus making it difficult to delineate a particular effect.[17]

Biochemical studies of carbohydrates are complicated by the fact that the interactions are often weak. Efficient binding relies on multivalent interactions[18] that are experimentally more difficult to measure. Carbohydrate heterogeneity and the cross-reactivity of sugar binding proteins require large numbers of carbohydrate ligands to be screened. High affinity lock-and-key fit, common for protein–protein binding, is rare and complicates experiments.

To overcome or circumvent these challenges, novel tools for glycomics have been developed,[3,4] including carbohydrate microarrays that specifically address the needs studying carbohydrate interactions.

4. Carbohydrate microarrays

Carbohydrate microarrays consist of sugars (Fig. 1) that are attached to a surface in a spatially defined and miniaturized fashion.[19–23] A spacer between the sugar and the surface ensures that the binding partner can gain access to the immobilized carbohydrate. The microarray format minimizes the amount of carbohydrate needed for each binding experiment and makes most out of the precious material. The dense presentation of the sugars on the surface mimics the situation encountered on cell surfaces that allows for multivalent interactions of relatively weak binding sugars. Carbohydrate microarrays have become a standard research tool to investigate sugar interactions within the past five years.

4.1 Carbohydrate microarray fabrication

Pure carbohydrates are required for their immobilization on a chip surface. Still, access to carbohydrates remains the major bottle-neck for the production of carbohydrate microarrays. The carbohydrates can be either isolated from natural sources or chemically synthesized. Carbohydrate isolation is a difficult process that often yields only low quantities of the desired structure. Due to carbohydrate heterogeneity, isolated oligosaccharides are often not completely pure and interference of the impurities cannot be ruled out. Isolated structures typically have to be equipped with a linker that consists of a spacer and a functional group for attachment to the array surface.

For chemically synthesized carbohydrates, linker incorporation is ideally a part of the total synthesis strategy. The synthesis relies on the sequential coupling of appropriately protected carbohydrate building blocks.[3,9] Following oligosaccharide assembly, all protective groups are removed and the linker is either installed or liberated, if it was present in protected form during the synthesis. Bioinformatics studies revealed that a large portion of the mammalian glycome can be chemically accessed from less than 40 carbohydrate building blocks.[13] Based on these building blocks, automated oligosaccharide synthesis[9,24] is beginning to accelerate access to defined carbohydrates. Production of oligosaccharides in days instead of months boosts the number of available carbohydrate structures. Despite the challenges associated with oligosaccharide synthesis, chemically prepared sugars guarantee pure materials and increase the structural diversity of sugars.

Both isolated and synthetic carbohydrates can be further modified enzymatically or chemically[3] to increase structural diversity. Enzymatic transformations of sugars immobilized on the surface of microarrays is feasible.[23]

A variety of microarray formats exist that utilize different surfaces and immobilization chemistries[19,21] (Fig. 2). Most of these reactions allow coupling of the carbohydrates to other surfaces and molecules. This enables easy follow-up investigations, including surface plasmon resonance (SPR), fluorescent imaging, and cellular assays, that validate the microarrays results and expand the knowledge gained.

The first carbohydrate microarrays consisted of sugars coupled to polystyrene microwells in resemblance of ELISA assays. Non-covalent attachment relied on the binding of biotinylated sugars to streptavidin,[25] or direct immobilization

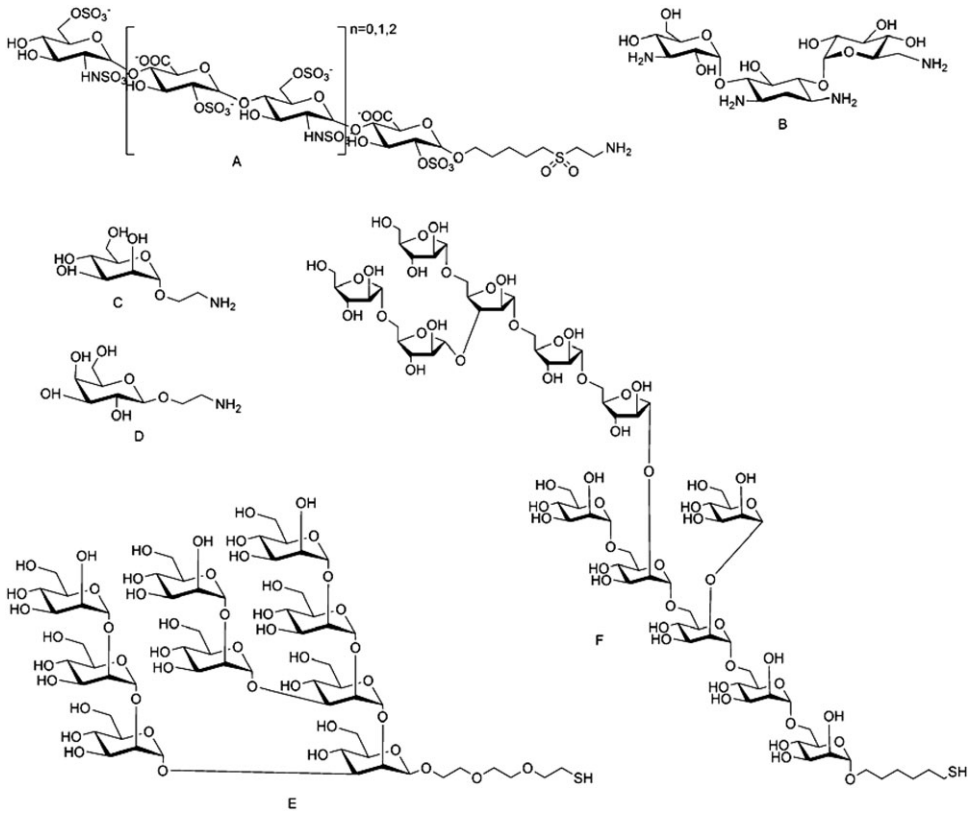

Fig. 1 Selection of carbohydrate compounds printed and used for carbohydrate microarrays (A: heparin oligosaccharide, B: aminoglycoside, C: mannoside, D: galactoside, E: nonamannoside, F: arabinomannoside).

of unmodified polysaccharides or lipidated sugars[26] to microtiter plates. Covalent immobilization *via* the formation of amides,[27] squarates,[28] cyanochloride coupling of amines,[29] and the [3 + 2]Huisgen-cycloaddition have been exploited.[19] Amide bond formation has been used to immobilize glycosylasparagine residues that were purified from glycoproteins.[30] Binding partners are exposed to the arrayed sugars and detected by correlating bound molecules to the colorimetric read-out of conjugated enzymes. Alternatively, fluorescence based detection can be used.[25] This system is readily compatible with rather inexpensive and broadly available ELISA equipment. Few sugars can be tested simultaneously and relatively large quantities of carbohydrates are needed for immobilization.

Other carbohydrate microarrays are based on the non-covalent attachment of neoglycolipids (NGLs) printed on nitrocellulose or poly(vinylidenefluoride) (PVDF) sheets similar to Western blots.[31] Molecules that bound to the arrayed sugars were detected by chemiluminescence in case of nitrocellulose or by fluorescence for PVDF. The technical hurdles for this approach are relatively low, but the detection limits are rather high and the number of sugar ligands that can be tested in parallel is limited.

The dominating carbohydrate microarray format relies on the attachment of sugars to microarray glass slides.[32,33] The glass slides are sometimes covered with a layer, matrix or gel-like structure such as dextran or BSA, that allows the sugars to be coupled into a three—rather than a two-dimensional environment that can improve immobilization and binding capacity, and stabilize the bound probes.[34] Some arrays are produced by non-covalent attachment of neoglycolipids to nitrocellulose covered glass slides[35] or fluorous-tagged carbohydrates to fluorinated slides;[36] however, most arrays consist of sugars that are covalently coupled to the microarray surface[33,37] (Fig. 3). Reliable, high yielding reactions have been used for carbohydrate attachment.[19,38,39] Typically, a reactive group is installed on the glass slide before the sugar bearing a

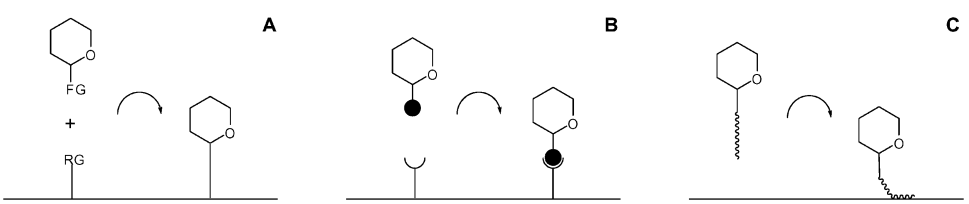

Fig. 2 *Overview of carbohydrate attachment to microarrays*: Covalent coupling to the surface using a reactive group (RG) and a corresponding functional group (FG) (A), non-covalent coupling using a receptor–ligand interactions (B), and non-covalent attachment using adsorption to the surface (C).

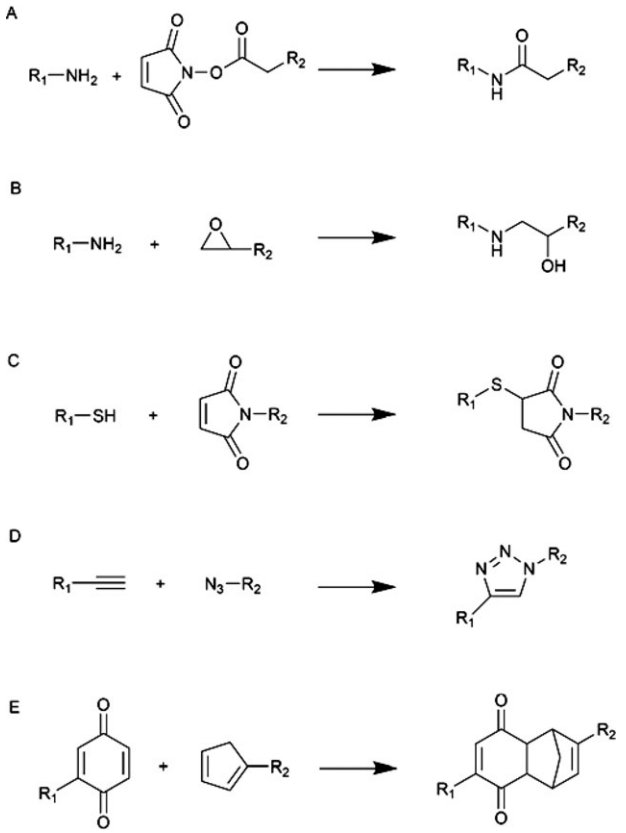

Fig. 3 *Overview of covalent coupling chemistries*: NHS-ester with amines (A), epoxides with amines (B), maleimides with thiols (C), alkynes with azides (D), and Diels–Alder cycloaddition (E).

compatible functional group is printed onto the reactive surface. The choice of the reactive group mainly depends on the synthesis strategy of the carbohydrate that is to be immobilized. The coupling reaction should be fast, specific and high yielding. The group should not interfere during the synthesis and should not react with other groups present on the carbohydrate. An amine reactive group is less suitable for coupling if the sugar itself contains amino-sugars such as glucosamines. Fast reactions are preferable, since reactions with surfaces are significantly slower than in solution. Thiols and maleimides,[37,40] amines and *N*-hydroxysuccinimide[33,41] or epoxides,[42] as well as azides and alkynes[43] or photoreactive groups[44] have been coupled. The sugars are printed onto the reactive slide using automated arraying robots. Incubation may range from several hours to days in order to complete the immobilization reaction.[25,45] A typical microarray spotter generates spots of approximately 200 μm in diameter by printing 1 nL.[37]

Interactions with the immobilized sugars are mostly detected using fluorescence. The fluorescent dye is either directly attached to the binding partner or indirectly *via* a fluorescently marked labeling agent. A microarray slide scanner is used for readout.

The glass slide based chips are true microarrays as hundreds of different carbohydrates can be coupled and tested on a single glass slide. Little substrate is needed and the detection is very sensitive. Thus, the glass slide approach has been used most often recently.

4.2 Conducting microarray experiments

Carbohydrate microarrays have been applied to screen the interactions of proteins,[31] RNA,[46] whole cells[47,48] and viruses[33] with carbohydrates. Mostly, carbohydrate binding proteins, so called lectins, have been tested.[25,45] Either labeled lectins or bound detection proteins were used for the read-out. The proteins are incubated on the microarrays to allow them to bind to the exposed carbohydrates before unbound proteins are washed from the surface. If necessary, antibodies or tag binding proteins are incubated subsequently in a similar fashion (Fig. 4).

Binding of RNA to carbohydrate microarrays were measured using either fluorescently labeled RNA[46] or by staining the bound RNA with dyes such as SYBR Green. Whole cells were stained with cell permeable nucleic acid dyes prior to incubation[48] or after binding to the slide using fluorescent dyes.[47] Whole viruses bound to microarrays were detected by incubating antibodies against proteins present on the virion in subsequent steps.[33]

Following incubation, the slides are centrifuged to dryness and scanned with a microarray scanner. The fluorescence intensities indicate the amount of ligand bound to the chip. PVDF membranes are directly scanned after fluorescent staining. Nitrocellulose membranes bearing enzymes for detection are overlaid with an appropriate substrate solution. For chemiluminescent detection, the sheets are scanned using chemiluminescence detection systems or are exposed to X-ray films. In case of microplate-based systems, the substrate solution is filled into the well after the incubation. After the reaction is stopped, the absorbance is measured spectroscopically using an ELISA reader. Alternatively, fluorescence detection methods can be used. In all cases, many binding events are measured that compare binding to the different carbohydrates arrayed on the chip.

5. Applications of carbohydrate microarrays

Soon after the first proof-of-principle arrays had been constructed and used, the focus turned to applications addressing

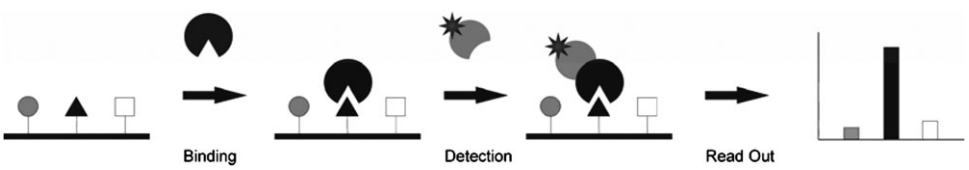

Fig. 4 Conduct of microarray experiments exemplified for protein binding. Binding of the protein to the arrayed sugars, binding of the fluorescently labeled detection protein, read out by a fluorescence scanner and analysis.

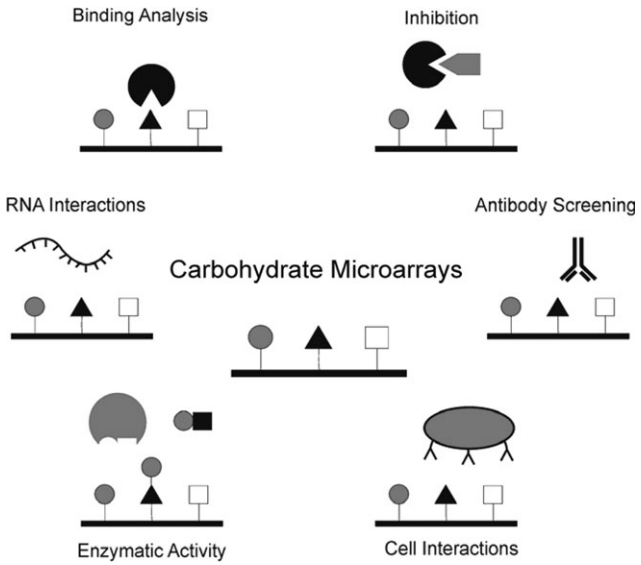

Fig. 5 Overview: Application range of carbohydrate microarrays experiments.

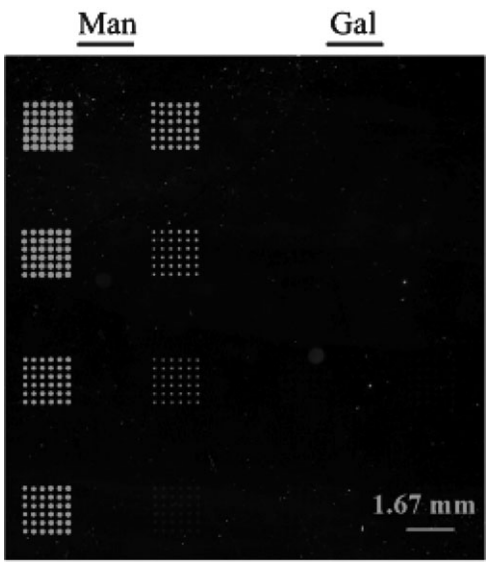

Fig. 6 Concanavalin A (a mannose binding lectin) binds specifically to mannose coupled on microarrays.[37] Reprinted from ref. 37 with permission. Copyright 2004, Wiley-VCH.

glycomics research (Fig. 5). Initially and still predominantly, the carbohydrate ligand specificity for carbohydrate binding molecules has been assessed.[31–33] Potential binders are added to the sugars on the microarray surface and the binding intensities are measured (Fig. 6). Sugar binding preferences can be determined by comparing the spot intensities. Protein–sugar interactions have been thoroughly established and provided valuable information regarding carbohydrate action *in vivo*. Binding preferences of different proteins were compared to begin to establish structure–function relationships for mutants or closely related proteins.

Carbohydrate microarrays bearing up to three hundred different sugar ligands are used to screen for unknown binding partners.[49] High affinity ligands identified by this approach may help to reveal the biological function of the carbohydrate binding protein. Antigenic carbohydrate structures can be identified and valuable information to design carbohydrate vaccines can be obtained. By dividing the array surface in wells of sugar ligands, sera can be screened for antibodies binding to carbohydrates in a high throughput manner.[50] These applications of carbohydrate microarrays showcase the rapid screening of many interactions to tackle the complexity and heterogeneity of the glycome.

Screening for inhibitors of carbohydrate-mediated interactions and determination of IC_{50} values can be performed by co-incubation of the binding molecule with an inhibitor.[51,52] In addition, kinetic constants can be calculated using carbohydrate microarrays.[53,54] Binding intensities at various dilutions are measured and kinetic constants can be determined. The action of enzymes modifying carbohydrates can be investigated using carbohydrate microarrays in a high-throughput manner[53,55,56] to analyze biosynthetic pathways of sugars by investigating specific actions of enzymes of the glycosylation machinery.

Since whole cells[47,48] and viruses[33] bind to carbohydrates on microarrays, sugar interactions of an entire organism can be determined without purifying the carbohydrate-binding proteins. Carbohydrate binding bacteria can be detected in crude mixtures and isolated for detailed examination.

5.1 Carbohydrate microarrays as research tools

Microtiter assays presenting carbohydrates *via* immobilized glycoproteins, whole cells or chemically linked synthetic carbohydrates[27] have been occasionally used in glycomics research for several decades, but these arrays displayed only few sugars and/or mixtures of polysaccharides in low density. Starting in 2002, the groups of Wang,[35] Mirksich,[51] Park,[40] and Fukui[31] presented carbohydrate microarray formats that overcame these problems allowing for the facile, versatile, high-throughput investigation of specific sugar interactions.

Amongst these new microarray formats, a neoglycolipid microarray printed onto nitrocellulose or PVDF membranes contained 95 different carbohydrates.[31] Interactions of nine well-known carbohydrate binding proteins, including antibodies, cytokines and selectins, with the arrayed sugars were evaluated. Binding was detected using either fluorescence or an enzymatic reporter reaction. Concentration dependent binding was demonstrated and the proteins showed the same binding pattern as previously determined.

Using fluorescence detection, ten different proteins, including plant lectins, human carbohydrate binding proteins, antibodies, bacterial and viral glycan binding proteins, and one whole virus were tested against 200 arrayed sugars.[33] Several new interactions were identified due to the large number of sugars on the array. A detailed binding pattern for each protein was established, including fine differences in specificity. These experiments proved that carbohydrate microarrays are suitable for the high throughput investigation of most carbohydrates.

The new carbohydrate microarray system was exploited to analyze glycan dependent interactions of two HIV-1 envelope proteins, gp120 and gp41[32] (Fig. 7). These proteins initiate the uptake of the virus into the host cell. Blocking proteins can

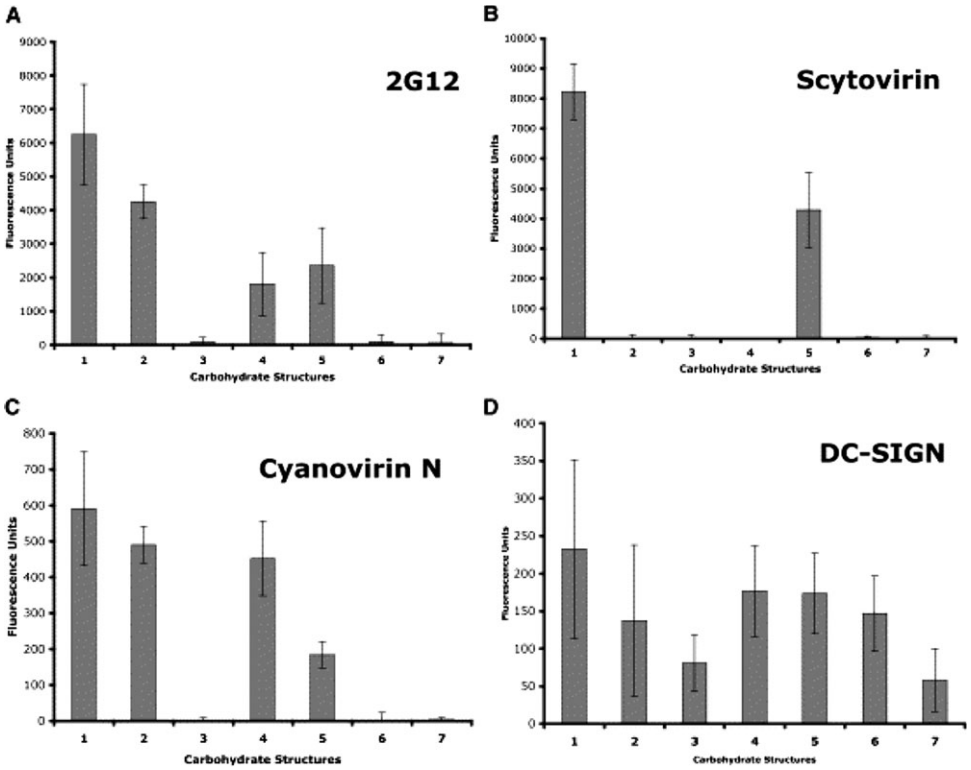

Fig. 7 Binding patterns of different mannose binding proteins.[32] Reprinted from ref. 32 with permission. Copyright 2004, Elsevier.

prevent rapid spreading of the virus by inhibiting interactions of the glycoproteins and stop the internalization of the virus. Glycan dependent interactions of proteins including the potential blocking proteins cyanovirin-*N*, scytovirin, the antibody 2G12, and DC-SIGN, a mannose binding receptor, with gp41 and gp120 were demonstrated. A carbohydrate microarray of high-mannose compounds was fabricated to analyze the binding patterns of the proteins.

In another study, a high affinity carbohydrate ligand for sialic acid-binding immunoglobulin-like lectin 8 (siglec-8) was identified using microarrays.[49] Siglec-8 is a protein expressed on a subset of immune cells and a member of the siglec family that comprises sialic acid binding proteins expressed on cell surfaces. Prior to this study, little was known about the function and mode of action of siglec-8 aside of a preference for sialic acid bearing compounds. By screening a microarray containing 172 carbohydrates, including 40 sialylated sugars, the binding preferences of siglec-8 were examined revealing one high affinity binding sugar. Binding was confirmed using SPR and dilution series of carbohydrates printed on microarrays, proving the high specificity of siglec-8 for 6′-sulfo-sLe^x, the identified ligand. To understand the function of lectins, it is vital to identify carbohydrate ligands of these proteins. Carbohydrate microarrays will be of great help to identify binding partners for the many lectins with no known high affinity ligands, amongst them several other siglecs. Binding specificities of five siglecs (human siglec-7, -8, -9, and murine siglec-2 and F) were determined using carbohydrate microarrays bearing 190 different carbohydrates.[57] Each siglec exhibited a specific binding pattern. By focusing on sulfated and sialylated carbohydrates, it was further revealed that sialylation is a prerequisite for binding of siglecs to most carbohydrates regardless on their state of sulfation. Sulfation leads either to increased or decreased binding of the siglecs, depending on the carbohydrate and protein without a general pattern. Thus, sulfation proved an important modulator of siglec–sugar interactions.

Carbohydrate microarrays were also used to investigate glycosyltransferases. The action of fucosyltransferase on LacNAc-residues coupled to a microarray surface was exploited to screen for suitable inhibitors.[55] The transferase was incubated on the microtiter array in the presence of GDP-fucose and the fucosylation of the coupled LacNAc-residues was measured by incubation with the fucoselectin from *Tetragonolobus purpureas*. Putative inhibitors were added to the enzyme solution prior to the incubation on the slide. Several inhibitors were identified. The substrate specificities of galactosyl-[53] and sialyltransferases[56] were examined in a high throughput manner using carbohydrate microarrays. The galactosyltransferase was incubated on the array in the presence of the substrates. To detect activity, the arrays were washed and incubated with an appropriate plant lectin that binds the newly added residue, but not unaltered carbohydrates. For sialyltransferases, the array was incubated with the enzyme and a biotinylated sugar donor. Successful transfers were detected by incubation with fluorescently labeled streptavidin.

Glycosaminoglycans are a large class of polysaccharides that consist of disaccharide repeating units containing glucosamine.[1] Depending on the type of the repeating unit and further modifications, these sugars are divided into subclasses including hyaluronan, chondroitin sulfate, keratin sulfate, dermatan sulfate and heparane sulfate/heparin. Proteoglycans

are part of the extracellular matrix and consist of heavily sulfated glycosaminoglycans attached to proteins. The complexity of glycosaminglycan chains is high due to the sulfation patterns. Glycosaminoglycans interact with a host of proteins, including growth factors, proteases, cytokines, chemokines, and cell adhesion molecules. However, little is known about the glycosaminoglycan motifs these proteins recognize. Better understanding would be an important step towards elucidating the biological role of glycosaminoglycan–protein interactions.

Heparin and heparane sulfate are the most prominent and complex members of the glycosaminoglycan family.[8] They consist of highly sulfated repeating units of glucosamine and glucuronic acid that is often epimerized to iduronic acid. In contrast to all other glycosaminoglycans, heparin is secreted as a soluble ligand by mast cells and it mainly regulates the blood coagulation pathway. Exploiting this action, purified heparin is a widely used anti-coagulant. In contrast to heparin, heparane sulfate is present on most cell types and forms an essential part of extra-cellular matrices in the human body, it consists of longer sugar chains that are attached to proteins, and it is less sulfated and modified. Heparane sulfate interacts with many proteins, thereby mediating or modulating their effects. However, heparin interacts and influences the proteins binding to heparane sulfate and *vice versa*. This is one of the major reasons for the heavy side effects of heparin as a drug.

Little is known about the specific interactions of most proteins with heparin and heparane sulfate with regard to the modification, especially sulfation, patterns. Heparin microarrays comprising glycosaminoglycans of different length and different modification patterns were generated.[41] The heparin microarrays bearing chemically synthesized heparin oligosaccharides were used to define the binding patterns of fibroblast growth factor (FGF)1, FGF2 and FGF4.[58] Thereby, specific binding patterns and differences were established. The microarrays were used to investigate synthetic activators of FGF2 and to determine their IC_{50} values.[52] SPR sustained the array results. Heparin microarrays were used to examine the binding pattern of chemokines, signaling molecules that lead to the recruitment of leukocyte subsets to the site of inflammation.[59] The chemokines showed different affinities for the carbohydrates arrayed.

Another glycosaminoglycan array was used to study interactions with growth factors and chemotatic proteins.[60] Purified and chemically modified glycosaminoglycans were non-covalently attached to polylysine covered microarrays. The binding pattern of proteins including different fibroblast growth factors and chemotatic proteins were determined and showed that several of these proteins act in a sulfation dependent manner.

A set of chondroitin sulfate oligosaccharides bearing these different sulfation patterns was chemically synthesized and printed onto microarrays to assess the binding of the growth factor midkine, the brain-derived neurotrophic factor (BDNF) and FGF1.[61] Midkine and BDNF bind one chondroitin sulfate subtype, whereas FGF1 did not interact with any chondroitin oligosaccharide present on the chip. The chondroitin sulfate oligosaccharides were analyzed in growth assays, with the best binders being the most active compounds.

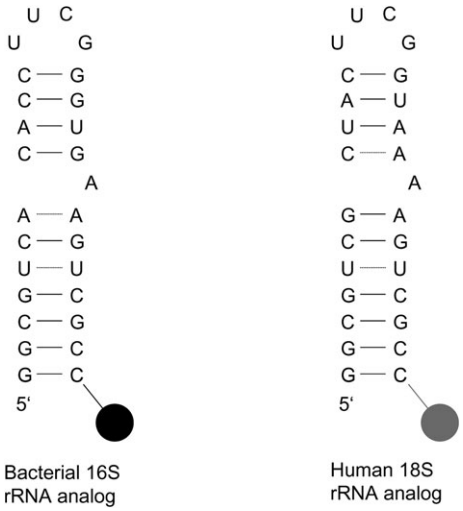

Fig. 8 Mimics of the ribosomal RNA labeled with fluorescent dyes at the 3′ end for detection.[46] Reproduced from ref. 46 with permission. Copyright 2004, Wiley-VCH.

The chondroitin microarrays were also used to analyze binding of TNF-α,[62] a major inducer of inflammation to show that TNF-α specifically interacts with chondroitin sulfate bearing a distinct sulfation motif. The finding helps to define the spatial action of TNF-α in organisms.

Aminoglycosides are a class of broad-spectrum antibiotics that bind to the 16S rRNA of bacteria, thereby inhibiting the bacterial protein translation. However, aminoglycosides also bind to host cell proteins, including DNA polymerase and phospholipase C, and these interactions are thought to cause many of the severe side effects known. A set of aminoglycosides was attached to microarrays and their interactions with a bacterial 16S rRNA mimic, a human 18S rRNA mimic (Fig. 8) and RNA from *Candida albicans*, a potential drug candidate, were measured.[46] Binding of DNA polymerase and phospholipase C to the aminoglycosides was also assessed. Comparing these data aided the selection of antibiotics that strongly interact with the bacterial 16S rRNA, but bind only poorly to the host cell proteins and rRNA, thus lowering side effects. The aminoglycoside arrays system was used to assess resistance mechanisms against aminoglycosides,[63] a growing problem. Pathogens can become resistant by expressing acetyltransferases that acetylate aminoglycosides to block their interaction with ribosomal RNA. Binding of two bacterial acetyltransferases to the arrayed compounds was measured to identify aminoglycosides that are less prone to resistance.

Interactions of whole human and chicken cells with carbohydrate microarrays have been analyzed.[47] Chicken hepatocytes bound to GlcNAc-terminated glycans, while human CD^+ T cells adhere to sialylLe^x bearing carbohydrates on glass slide microarrays. This technique opens the possibility to screen and test for carbohydrate-specific cell interactions without the need to purify the proteins of interest.

5.2 Carbohydrate microarray as diagnostic tools

Microarrays offer an attractive platform for diagnostic applications since many binding events can be screened in parallel. Bacterial adhesion to carbohydrate microarrays was

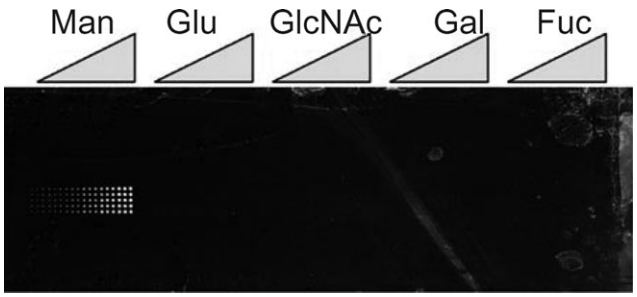

Fig. 9 Bacteria (*E. coli*) specifically bound to mannose on carbohydrate microarrays.[48] Reprinted from ref. 48 with permission. Copyright 2004, Elsevier.

investigated for *E. coli*, as the sugar based attachment renders these bacteria into harmful pathogens.[48] It was demonstrated that *E. coli* bacteria bearing a receptor protein (Fim H) that interacts with mannose, bind specifically to mannose on carbohydrate microarrays while bacteria lacking Fim H do not bind (Fig. 9). The microarrays served to test inhibitors of binding and to measure IC_{50} values. A multivalent mannose probe was the most potent inhibitor and underscored the importance of multivalency for carbohydrate interactions. These microarrays may also be used as diagnostic test to detect pathogens. Harmful adherent strains and non-adherent bacteria can be readily distinguished with a detection limit of 10^6 bacteria even in blood. Bacteria that bind to the microarray spots were cultured and prepared for further testing. Thereby, bacteria that attach to carbohydrates can be isolated from crude samples for further examination. These findings open the possibility to develop an easy and cheap diagnostic test to detect pathogens.

Carbohydrate microarrays constitute an excellent platform to test sera for antibodies that bind to specific sugar structures. The high throughput format enables to screen large numbers of sera in parallel. In a preliminary study,[35] binding of specific antibodies to dextrans, glucose polymers from certain bacteria, non-covalently attached to nitrocellulose coated glass slides was examined and it was demonstrated, that the antibodies had different binding preferences for the sugars. To investigate this system for analyzing human antibody repertoires, 48 polysaccharides were printed onto microarrays. A set of 20 human sera was investigated and several antibodies binding to the carbohydrate compounds were identified. The microarray system was further used to analyze cross-reactivity of carbohydrate interactions and it was demonstrated that some monoclonal antibodies bound to other carbohydrate epitopes than expected. This cross-reactivity of the antibodies was confirmed by staining of tissue sections.

A microarray bearing *Salmonella O*-antigens was fabricated to assess antibody levels in human sera and to distinguish between infections with different strains.[50] The binding patterns of known polyclonal antibody sera raised against certain *Salmonella* strains were measured and validated using a monoclonal antibody. Analysis of human sera of salmonellosis patients showed that antibodies against specific carbohydrate antigens were present and different sugars were recognized depending on the *Salmonella* subtype. The results proved that carbohydrate microarray tests can be used to detect and distinguish infections, to analyze antigenic sugar structures and to evaluate carbohydrate based vaccines.

Detailed binding preferences of hemagglutinins were assessed using carbohydrate microarrays.[12] Hemagglutinin proteins are present on the capsule of influenza viruses and mediate the uptake into the host cell by binding to sialylated carbohydrates. Human H3N2 hemagglutinins preferentially bind α2–6 linked sialic acid bearing oligosaccharides whereas the avian H5N1 proteins prefer α2–3 linkages. In humans, α2–6 linked sialic acid moieties are found on epithelial cells in the upper respiratory tract where they are readily accessible for inhaled viruses. In contrast, α2–3 linkages are found on epithelial cell in the respiratory tract of birds. Thus, these different binding preferences of the hemagglutinin proteins contribute to generate the host species barrier.

Using carbohydrate microarrays bearing 200 carbohydrates including many sialylated structures, the binding pattern of several human H3N2 and avian H5N1 hemagglutinins were analyzed. The known general specificity of human hemagglutinins for α2-6 linkages and avian proteins for α2-3 linkages was confirmed. In addition, the fine binding specificities of single hemagglutinin proteins were unraveled. It was demonstrated that each protein has characteristic binding patterns. Thus, a test system to identify and analyze influenza viruses based on their binding preferences should be feasible. Since whole influenza viruses also bind to carbohydrate microarrays in a sugar specific manner, this method may be used for the analysis and detection of viruses derived from infected specimens. Current tests to characterize influenza virus strains last several days. Using carbohydrate microarrays, tests that are more sensitive and faster than current tests might be in reach to detect avian influenza strains in early stages of epidemic infections and to track changes in binding specificities suggesting dangerous mutations in avian influenza strains.

6. Summary

Methods for the construction of carbohydrate microarrays, their use in binding experiments and applications of carbohydrate microarrays are reviewed in this paper. These arrays have become indispensable tools for investigations in the glycomics field. Relatively small amounts of valuable materials are consumed; multivalent interactions that mimic the natural presentation of carbohydrates on the cell surface can be probed. Applications in a range of research and diagnostic settings screened interactions of carbohydrates with proteins, RNA, whole cells and viruses. Sugar binding preferences can be readily assessed, screening for high affinity ligands, and measuring kinetic constants is possible. More medically relevant experiments include screening of sera for glycan specific antibodies, investigation of cell adhesion and specific detection of pathogens and viruses. Carbohydrate microarrays quickly have become a standard research tool. In years to come, medical applications to identify carbohydrate markers and to detect these markers in patient samples will become an important area of this technology.

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