Carbohydrate microarrays — a new set of technologies at the frontiers of glycomics

Ten Feizi[*†], Fabio Fazio[‡], Wengang Chai[*] and Chi-Huey Wong[‡§]

Carbohydrate microarray technologies are new developments at the frontiers of glycomics. Results of 'proof of concept' experiments with carbohydrate-binding proteins of the immune system — antibodies, selectins, a cytokine and a chemokine — and several plant lectins indicate that microarrays of carbohydrates (glycoconjugates, oligosaccharides and monosaccharides) will greatly facilitate not only surveys of proteins for carbohydrate-binding activities but also elucidation of their ligands. It is predicted that both naturally occurring and synthetic carbohydrates will be required for the fabrication of microarrays that are sufficiently comprehensive and representative of entire glycomes. New leads to biological pathways that involve carbohydrate–protein interactions and new therapeutic targets are among biomedically important outcomes anticipated from applications of carbohydrate microarrays.

Addresses
[*]The MRC Glycosciences Laboratory, Imperial College London, Northwick Park & St Mark's Hospital Campus, Watford Road, Harrow, Middlesex HA1 3UJ, UK
[†]e-mail: t.feizi@imperial.ac.uk
[‡]Department of Chemistry and Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10440 North Torrey Pines Road, La Jolla, California 92037, USA
[§]e-mail: wong@scripps.edu

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Abbreviations

Con A	concanavalin A
ELISA	enzyme-linked immunosorbent assay
NGL	neoglycolipid
RRV	relative reactivity value
TLC	thin-layer chromatography

Introduction

There is an increased awareness, post genome, of glycosylation as a potentially important form of post-translational modification of proteins [1]. At the same time, increasing numbers of receptors are being characterized that operate through binding to specific oligosaccharide sequences on glycoproteins, glycolipids and polysaccharides [2–7]. Such receptors include effector proteins involved in the folding of nascent proteins, the subcellular targeting of enzymes, mechanisms of infection (microbe–host interactions), and mechanisms of inflammation and immunity (both innate and adaptive). The pinpointing and characterization of carbohydrate ligands remains, however, one of the most challenging areas of cell biology [8]. This is because the range of carbohydrate structures is enormously diverse and ligands that are oligosaccharides cannot be readily cloned; each is the product of multiple glycosyltransferases and other modifying enzymes. Thus, conventional approaches to carbohydrate ligand discovery are cumbersome and there is a great need for sensitive, high-throughput technologies that perform analyses of carbohydrate–protein interactions in order to detect and characterize the oligosaccharide sequences within glycomes that are bound by carbohydrate-recognizing proteins. Microarray approaches, analogous to those developed for DNA [9] and being developed for proteins [10], are ideal for addressing this need. Only small amounts of product are required for fabricating microarrays and many compounds can be screened in parallel in a single operation.

For the definitive assignment of the ligands of carbohydrate-binding proteins, it is essential to have pure monosaccharides and oligosaccharide sequences. As modern techniques analogous to those of molecular biology and recombinant protein expression cannot be applied to generate oligosaccharides for microarrays, multiple strategies are needed to access oligosaccharides for arraying, namely synthetic approaches (chemical or enzymatic syntheses) and isolation from natural sources. Among other aspects for consideration are the composition of the solid support, the means of attachment of carbohydrates to the support (covalent or noncovalent) and the refinement of analytical systems to render them suitable for the characterization of the immobilized saccharides. We briefly review here current approaches to the synthesis and isolation of oligosaccharides, before highlighting developments in the emerging field of carbohydrate microarrays.

Carbohydrate sources for the fabrication of arrays

Synthesis of oligosaccharides

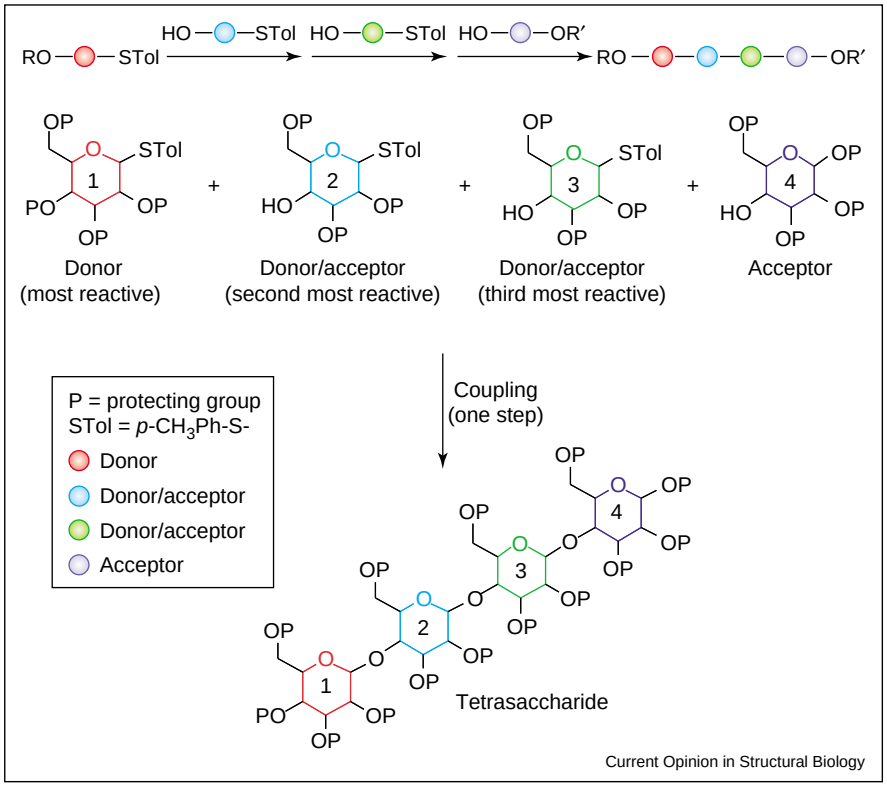
Unlike proteins and nucleic acids, oligosaccharides are difficult to synthesize chemically. This is because some oligosaccharide chains are linear, others are branched, the monosaccharide building blocks are in α or β anomeric configurations, and adjacent monosaccharides are linked via different carbon atoms in their sugar rings. For these reasons, multiple selective protection and deprotection

steps are required for the hydroxyl groups of monosaccharides during chemical synthesis of oligosaccharides; the manual synthesis of oligosaccharides is a major undertaking. Nevertheless, syntheses of a considerable number of complex oligosaccharides have been achieved (e.g. [11–17]), and the products have been invaluable in studies of the specificities of antibodies and other carbohydrate-binding proteins of the immune system.

The solid-phase synthesis approach has the advantage of avoiding intermediate isolation and purification steps. An automated solid-phase method that includes selective protection and deprotection steps has been introduced and applied to the synthesis of several glucose- and mannose-containing oligosaccharides [18]. An alternative approach to the synthesis of oligosaccharides is a programmable 'one-pot' approach, in which an oligosaccharide of interest is generated by the sequential addition of building blocks (thioglycosides) that are either fully protected or have one hydroxyl group exposed. These modifications confer different reactivity rates and anomeric configurations on glycosyl donors and acceptors (see [19] and references therein; Figure 1). It has been shown that the relative reactivity value (RRV) of a thioglycoside building block in the glycosidation reaction can be tuned in the presence of protecting groups; more than 200 building blocks, with RRVs ranging from 1 to 10^5, have been designed and synthesized. A computer programme called 'Optimer' has been developed to guide the selection of building blocks for the one-pot synthesis of a given oligosaccharide. If RRVs differ by more than 10^2, the desired glycosidic bonds will be formed by the sequential addition of building blocks in the order of the RRV values. Once the required building blocks with protecting groups are prepared, oligosaccharides can be synthesized in a short period of time (in minutes or hours, instead of days or months using traditional methods) using this programmable one-pot approach [20,21].

Besides chemical synthesis, oligosaccharides can be prepared by enzyme-catalyzed reactions [22]. In nature, there exist two classes of enzymes that can be harnessed to build oligosaccharides: glycosyltransferases and glycosidases. The former catalyze the formation of glycosidic

Figure 1



Schematic representation of a programmable one-pot approach to oligosaccharide synthesis; a linear 1,4-linked tetrasaccharide is depicted as an example. The constituent building blocks are classified into three species: the first sugar at the nonreducing end acts as the donor; the last sugar at the reducing end is the acceptor; all other building blocks that form the inner part of a complex (linear or branched) oligosaccharide are classified as donor/acceptor. Protecting groups (esters or ethers) determine the RRV of anomeric centers. Building blocks are added in the order 1 to 4 to obtain the tetrasaccharide.

bonds and the latter hydrolyze them. For laboratory synthesis, both classes of enzymes have been utilized (glycosidases in their reverse reactions) with advantages and shortcomings. The first and most important advantage of enzymatic synthesis of oligosaccharides is that substrates are used in their natural form. No protecting groups are needed to direct the regio- or stereo-specificity of glycosidic bond formation. Of the two classes of enzymes, glycosyltransferases work best, and products are formed in high yield and with excellent regio- and stereo-selectivity. The major drawbacks of enzymatic synthesis are, however, the limited availability of glycosyltransferases and their high cost. There have been promising applications of glycosidases to oligosaccharide synthesis after active site mutations to yield glycosynthases that can accept glycosyl fluorides for glycosylation [23].

Isolation of oligosaccharides from natural sources

Oligosaccharides with reducing termini are ideal for derivatization so that they can be immobilized. Free reducing oligosaccharides may be isolated from human or animal milk and urine, or they may be in the form of *N*-linked glycoprotein oligosaccharides released by the enzymes peptide-*N*-(*N*-acetyl-β-glucosaminyl)asparagine amidase (PNGase F) and endo-β-*N*-acetylglucosaminidase F (Endo F) [24], or by hydrazinolysis [25]. *O*-linked glycoprotein oligosaccharides may be released by mild alkaline hydrolysis [26] or hydrazinolysis [27]. Oligosaccharides may, if desired, be released from glycolipids by endoceramidase [28]. Oligosaccharide fragments can be obtained from proteoglycans and glycosaminoglycans by lyase digestion [29] or nitrous acid degradation [30], and, in the case of hyaluronic acid, also by hydrolase digestion [31]. Various chemical methods may be used to obtain oligosaccharide fragments from bacterial and plant polysaccharides; these include acid or alkaline hydrolysis, acetolysis and Smith degradation [32].

Reduced oligosaccharides (oligosaccharide alditols) can be manipulated chemically at the reduced end after mild periodate oxidation [33] to cleave the terminal open chain monosaccharide residue and create a reactive aldehyde for derivatization. Reduced oligosaccharides are typically obtained when *O*-linked glycans are released from glycoproteins by reductive alkaline hydrolysis [34–36]. Oligosaccharide alditols are also available when reduction is carried out, for example, before HPLC separation, to eliminate double peaks resulting from the resolution of α and β anomers at their reducing ends.

Multiple chromatographic steps are often necessary for the isolation/purification of oligosaccharides. These include gel filtration, weak and strong anion-exchange chromatography, thin-layer chromatography (TLC), normal-phase HPLC with an amine or amide column, and reversed-phase HPLC using a C18 or graphitized porous carbon column.

Arrays of monosaccharides and disaccharides

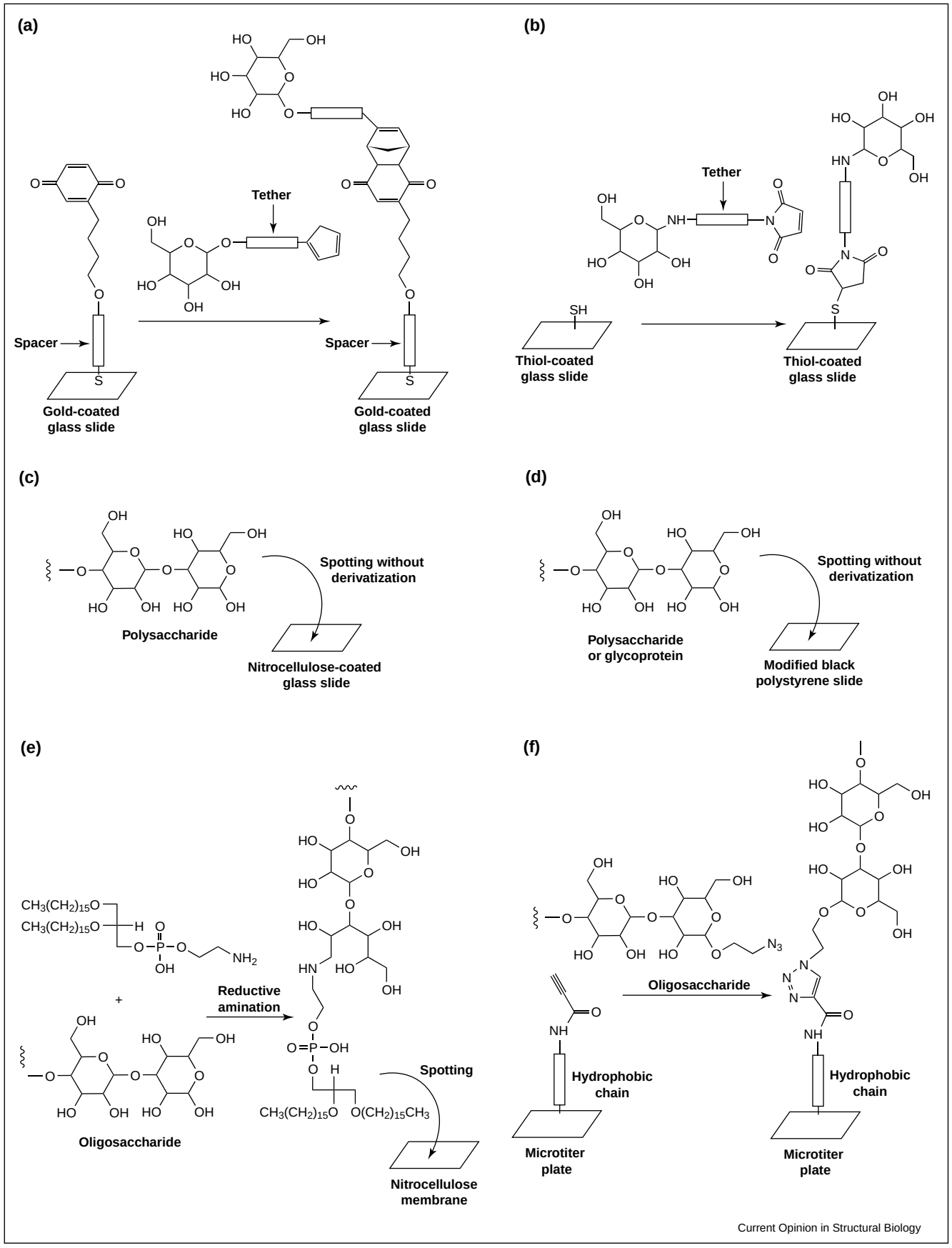
Mrksich and colleagues [37•] have described a microarray of ten monosaccharides on gold-coated glass slides. The monosaccharides were covalently immobilized by conjugation to self-assembling monolayers of alkenethiols on the gold surface (Figure 2a). The first step was to prepare monolayers consisting of two alkenethiols, one of which has a benzquinone group exposed. The monosaccharides, in the form of diene conjugates, are then applied as 1 μl spots (2 mM in water) and attached to the slides through the Diels–Alder cycloaddition reaction. This is a very high yielding process, often reagent free, and moisture and solvent tolerant, and is therefore ideal for the microarray format. The covalently immobilized monosaccharides were evaluated by profiling the binding specificities of five plant lectins, concanavalin A (Con A), *Benderia simplicifolia*, *Erythrinia cristalli*, *Ulex europeeus* and *Galanthus nivalis*, that are known to bind to different monosaccharides. Specific monosaccharide binding was observed for the five lectins, which were labeled fluorescently with rhodamine. Specific binding of Con A to arrayed mannose was also shown by surface plasmon resonance spectroscopy. In further experiments, the monosaccharide array was probed with the glycosyltransferase β-1,4-galactosyltransferase; it was shown that enzyme-mediated glycosylation of immobilized *N*-acetylglucosamine occurred in the presence of the donor substrate, UDP-galactose.

Shin's group [38•] has reported another approach to carbohydrate microarray fabrication. They used glass slides modified by thiol groups as solid supports. One monosaccharide, *N*-acetylglucosamine, and three disaccharides, lactose, cellobiose and maltose, in the form of glycosylamines, were converted into maleimide conjugates and then covalently bound to the glass surface by hetero-Michael addition reaction between the thiol group on the solid surface and the maleimide moiety of the sugar derivative (Figure 2b). The maleimide-conjugated carbohydrates (from 0.1 to 5.0 mM) were printed with a pin-type microarrayer on the slides at a spot size of ~100 μm and a pitch of 200 μm. Carbohydrate–protein interaction studies were performed with fluorescein-labeled plant lectins. The binding of the three plant lectins examined, Con A, *Erythrina cristagalli* and *Triticum vulgaris*, to the monosaccharide and the disaccharides was in accord with their known specificities.

Arrays of polysaccharides

Wang *et al.* [39••] described microarrays of polysaccharides and glycoproteins on nitrocellulose-coated glass slides. They used a high-precision robotic arrayer that was developed for cDNA and the spots were generated without derivatization (Figure 2c). The spot sizes were ~150 μm with a pitch of 375 μm. These were air dried to allow adsorption (noncovalent immobilization) onto the

Figure 2


(a)
Tether
Spacer
Gold-coated glass slide
Spacer
Gold-coated glass slide
(b)
Tether
Thiol-coated glass slide
Thiol-coated glass slide
(c)
Spotting without derivatization
Polysaccharide
Nitrocellulose-coated glass slide
(d)
Spotting without derivatization
Polysaccharide or glycoprotein
Modified black polystyrene slide
(e)
Reductive amination
Oligosaccharide
Spotting
Nitrocellulose membrane
(f)
Oligosaccharide
Hydrophobic chain
Microtiter plate
Hydrophobic chain
Microtiter plate
Current Opinion in Structural Biology

hydrophobic surface. The authors evaluated the extent of immobilization of fluorescein-labeled preparations of dextrans (ranging from 20 to 2000 kDa) and inulin (3.3 kDa) by arraying these on the coated slides and subjecting them to washing procedures. All were immobilized, although the efficiency of immobilization was dependent on molecular mass; the larger molecules were better retained than the smaller molecules. Nevertheless, the authors clearly showed the applicability of this array procedure to antigenic analyses of bacterial polysaccharides. They illustrated this, first, by using well-characterized monoclonal antibodies to different sequences on dextrans and, second, by using human sera to detect antibodies to different bacterial polysaccharides. Antibody binding was variously detected by color development with alkaline-phosphatase-conjugated secondary antibodies or by fluorescence using biotinylated secondary antibodies followed by Cy3-labeled streptavidin.

Willats *et al.* [40•] have worked with another type of microarray slide surface for polysaccharides — relatively hydrophobic black polystyrene produced with a physical surface modification that increases the surface area available for passive (noncovalent) adsorption (Figure 2d). The arrayed samples were derived from plant cell walls, and included complex neoglycoproteins, proteoglycans and polysaccharides known to have different patterns and degrees of methyl-esterification. These polysaccharides were directly applied as spots, ~50 pl, with a pitch of 375 μm, and were probed with previously characterized hybridoma antibodies and a phage-derived antibody. The predicted patterns of antibody binding were observed using Cy3-labelled secondary antibodies.

Arrays of oligosaccharides

The low mass and hydrophilic nature of most oligosaccharides preclude their direct noncovalent immobilization on solid matrices. To overcome this limitation, Feizi and colleagues [41••] have established a microarray system in which the oligosaccharides are linked to lipid. The oligosaccharides, containing two to twenty monosaccharide residues, were linked by reductive amination to the amino phospholipid 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine or its anthracene-containing fluorescent analogue. These neoglycolipids (NGLs) and, in addition, glycosylceramides were applied to nitrocellulose membranes (Figure 2e) by jet spray with a sample applicator (300 μm spots). It was shown that they could be robustly attached by noncovalent absorption. The oligosaccharide sequences were sourced from natural secretions — glycoproteins (*O*- and *N*-glycans), glycolipids, proteoglycans, polysaccharides, a whole organ (mammalian brain) — or from chemical synthesis. The carbohydrate-binding proteins investigated were known sequence-specific hybridoma antibodies, the E- and L-selectins, a chemokine (RANTES) and a cytokine (interferon γ). Binding was detected by colorimetric ELISA-type methods. It was shown that carbohydrate-binding proteins could single out their ligands, not only in arrays of homogeneous, structurally defined oligosaccharides but also in an array of heterogeneous *O*-glycan fractions derived from brain glycoproteins. The unique feature of this technology is that deconvolution strategies are included with TLC and mass spectrometry for determining the sequences of ligand-positive components within mixtures.

Wong's group [42•] has reported an alternative method for the fabrication of noncovalent microarrays of monosaccharides and oligosaccharides. Starting with allyl derivatives of galactose, the monosaccharide was linked via its anomeric position, under stereochemically controlled conditions, to aliphatic hydrocarbons (3–21 carbons in length). The polystyrene surface of 96-well microtiter plates was used as the solid support for the noncovalent attachment of the derivatives. When the saturated hydrocarbon chain was between 13 and 15 carbons in length, the monosaccharide was completely retained in wells after aqueous washing. Therefore, a saturated hydrocarbon with a 14-carbon chain was selected for conjugating several synthetic, neutral di- to hexasaccharide glycosides containing terminal galactose, glucose or fucose. All of these were stable after repeated washings and elicited the predicted binding signals with the lectins *Ricinus* B chain, Con A and *Tetragonolobus purpurea*. In another approach to fabrication of an array in a microtiter plate format, Wong and colleagues [43•] first noncovalently immobilized the long-chain (14-carbon) aliphatic alkyne on the plastic surface and then carried out *in situ* conjugation of the azide forms of galactose and several neutral and sialic acid containing di- to tetrasaccharides. The saccharides were conjugated by a 1,3-dipolar cyclo addition reaction between the azide and alkyne groups (Figure 2f). This method simplifies the preparation and purification of the lipid-linked oligosaccharides. The noncovalent attachment also allowed convenient characterization of the lipid-linked products by mass spectrometry, as well as the detection of lectin binding by color development. Using GDP-fucose and α-1,3-fucosyltransferase, fucosylation of sialyl-*N*-acetyllactosamine was carried out within wells. The process would be well suited to the high-throughput identification of enzyme inhibitors.

(Figure 2 Legend) Diagrammatic representation of the fabrication of carbohydrate microarrays. **(a)** Covalent immobilization on a gold surface by Diels–Alder reaction. **(b)** Covalent immobilization on a glass slide by hetero-Michael addition reaction. **(c)** Noncovalent polysaccharide adsorption without derivatization onto a nitrocellulose-coated glass slide. **(d)** Noncovalent polysaccharide and glycoprotein adsorption without derivatization onto a modified black polystyrene slide. **(e)** Noncovalent adsorption of NGLs onto a nitrocellulose membrane. **(f)** Noncovalent immobilization by hydrophobic interaction of a 14-carbon aliphatic chain with polystyrene wells.

Biotinylation of oligosaccharides and their attachment on streptavidin-coated surfaces is another means of noncovalent immobilization. The Glycomics Consortium (http://glycomics.scripps.edu), sponsored by the National Institutes of Health, uses this strategy to produce a synthetic carbohydrate microarray.

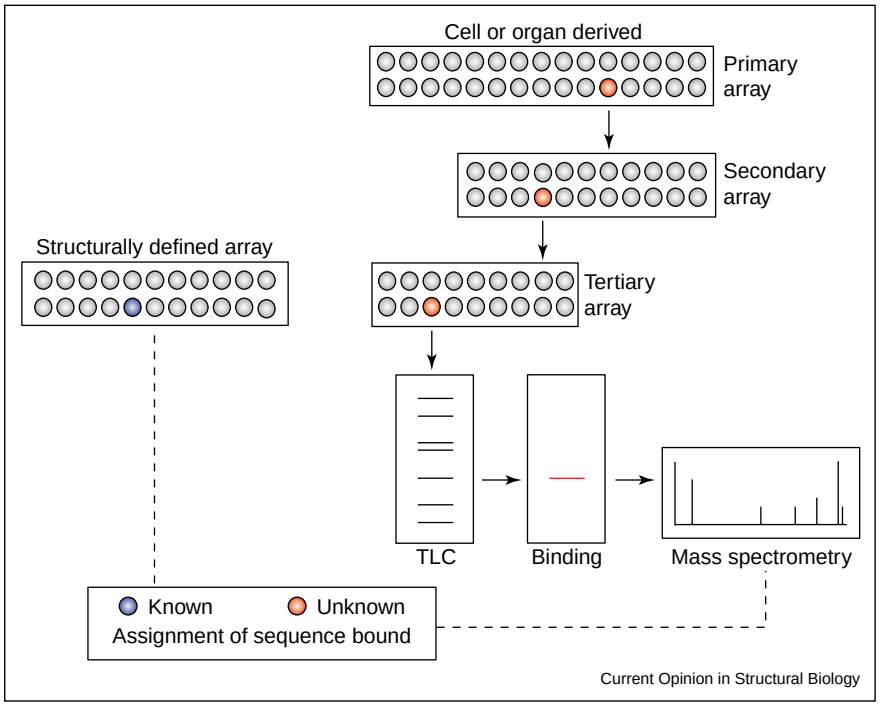
Careful comparisons of the various immobilization approaches will be required, as it has been observed that the relative intensities of binding signals elicited with biotinylated oligosaccharides thus immobilized may not always tally with those elicited with lipid-linked oligosaccharides [44].

Perspectives

The field of carbohydrate microarrays is in its infancy and further developments are anticipated, such as new fabrication strategies, new array surfaces and printing methods, further miniaturization of arrays and enhanced sensitivities of detection. The results of the several 'proof of concept' experiments reported thus far have been very promising, and it is envisaged that microarrays of oligosaccharides and also glycoconjugates will revolutionize surveys of proteins for carbohydrate-binding activities. Microarrays of polysaccharides and glycoproteins clearly have a place in serological studies, and also in the initial selection and workup of antibodies and carbohydrate-binding proteins. Microarrays of defined oligosaccharide sequences and orientations will be key to the assignment of the epitopes or ligands for carbohydrate-binding proteins.

Many saccharides can be examined in parallel for binding once they are arrayed. But the establishment of a microarray that is representative of a glycome will be a nontrivial undertaking. With the advances being made in chemical and chemo-enzymatic syntheses, increasing numbers of known oligosaccharide sequences will become available for incorporating into microarrays. It is foreseen that new methods will be developed for rapid diversity-oriented syntheses of oligosaccharides on nano- and micro-scales. Oligosaccharides from natural sources will be an important resource for microarrays for two reasons. First, it is currently impractical to synthesize in the laboratory the full complement of known sequences in animals, *O*- and *N*-glycans of glycoproteins, diverse sequences of glycosaminoglycans and glycolipids, and those of the glycoconjugates of bacteria, fungi and plants. Second, there are bound to be hitherto unknown oligosaccharide sequences in glycomes. The NGL principle for microarrays [41••,45•], which is based on an established technology for carbohydrate ligand discovery [46], has the potential to address challenges of the fabrication of oligosaccharide microarrays from particular glycomes. As depicted in Figure 3, the NGL approach is geared for generating tailor-made microarrays of oligosaccharides

Figure 3



Diagrammatic representation [45•] of microarrays of lipid-linked oligosaccharides incorporating deconvolution steps for the assignment of sequences recognized by carbohydrate-binding proteins. Depending on the complexity of the glycan populations, it may be necessary, after primary arrays, to fabricate subarrays or daughter arrays from ligand-positive parent spots.

from desired biological sources. At the same time, the technology encompasses structurally defined oligosaccharides isolated from natural sources, resorting to chemically synthesized sequences for those that are available in limited amounts from natural sources. It will be important to further refine the technology to have an unmodified (ring-closed) core monosaccharide residue or to include glycoprotein linkage region amino acids, as there are examples of carbohydrate-binding proteins that require the intact core sugar or flanking peptide for binding.

In conclusion, carbohydrate microarrays hold great promise as a high-throughput means of detecting the interactions of proteins with diverse oligosaccharide sequences of glycoproteins, glycolipids and polysaccharides. The findings should be key starting points for detailed characterizations *in vitro* and *in vivo*. For example, quantitative microwell binding and inhibition experiments would be required, such as those carried out for the selectins [47] and glycosyltransferases [43•]. These would lead to or be accompanied by structural studies, as with carbohydrate-recognizing proteins of the innate immune system [48,49]. There are also issues to consider such as the influence of specific carrier proteins on presentation and the availabilities of glycans for binding by particular proteins [50], as well as those displayed *in vivo* on cells. As more and more recombinant proteins of biomedical interest become available for investigation, the evaluation of their carbohydrate binding will be greatly helped by carbohydrate microarrays. The anticipated outcomes are new leads to biological pathways and new drug targets, namely carbohydrate mimics [51,52] that can therapeutically boost or inhibit protein–carbohydrate interactions.

Update

A paper in press from Glycominds Ltd describes a synthetic glycan array in 96-well format for the profiling of anti-glycan antibodies [53].

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The authors describe the fabrication of a carbohydrate array of ten monosaccharides by covalent attachment of the monosaccharides to gold-coated glass slides via linkers comprising self-assembling monolayers of alkanethiols. Five plant lectins tested are shown to bind to the arrayed monosaccharides in accordance with their known specificities. Lectin binding studies were extended to show that monolayers presenting arrayed *N*-acetylglucosamine are good acceptors for galactosylation by β-1,4-galactosyltransferase in the presence of UDP-galactose. Thus, carbohydrate chips have potential applications in examining enzymatic activities toward carbohydrate substrates (see also [43•]).

38. • Park S, Shin I: **Fabrication of carbohydrate chips for studying protein-carbohydrate interactions**. *Angew Chem Int Ed Engl* 2002, **41**:3180-3182.

A microarray is described comprising the monosaccharide *N*-acetylglucosamine and the disaccharides lactose, cellobiose and maltose, which are covalently attached via maleimide linkers to glass surfaces modified by thiol groups. Three plant lectins are shown to bind to the arrayed carbohydrates in accordance with their known specificities.

39. •• Wang D, Liu S, Trummer BJ, Deng C, Wang A: **Carbohydrate microarrays for the recognition of cross-reactive molecular markers of microbes and host cells**. *Nat Biotechnol* 2002, **20**:275-281.

This is the first published report of a carbohydrate microarray. The arrayed materials are 48 glycoproteins and polysaccharides, each immobilized by noncovalent absorption on nitrocellulose membranes. The microarrays are shown to be well suited to antigenic analyses with monoclonal antibodies and to the detection of antibodies to bacterial polysaccharides in human sera.

40. • Willats WG, Rasmussen SE, Kristensen T, Mikkelsen JD, Knox JP: **Sugar-coated microarrays: a novel slide surface for the high-throughput analysis of glycans**. *Proteomics* 2002, **2**:1666-1671.

The authors describe microarrays of proteoglycans, neoglycoproteins and polysaccharides known to have various degrees and patterns of methyl-esterification, derived from plant cell walls. Hydrophobic polystyrene slides with rough surfaces were used for noncovalent attachment of the carbohydrates. When probed with previously characterized monoclonal antibodies and a phage-derived antibody, the patterns of binding were found to be in accord with their known specificities.

41. •• Fukui S, Feizi T, Galustian C, Lawson AM, Chai W: **Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate-protein interactions**. *Nat Biotechnol* 2002, **20**:1011-1017.

Arrays of naturally occurring and synthetic oligosaccharides are described that are intended for generating large repertoires of immobilized oligosaccharides representing those derived from glycoproteins, proteoglycans, glycolipids and polysaccharides. The oligosaccharides were linked by reductive amination to an amino phospholipid to obtain NGLs, or they may be in the form of glycosylceramides, and attached to nitrocellulose membranes by noncovalent absorption. Some 30 structurally defined oligosaccharides and 65 oligosaccharide fractions were thus arrayed, and specific binding was shown with 8 sequence-specific antibodies and 4 other carbohydrate-binding proteins of the immune system. A deconvolution strategy is included with TLC and mass spectrometry for sequencing ligand-positive components within mixtures (see also [45•]).

42. • Bryan MC, Plettenburg O, Sears P, Rabuka D, Wacowich-Sgarbi S, Wong CH: **Saccharide display on microtiter plates**. *Chem Biol* 2002, **9**:713-720.

Conditions are established for fabricating, by noncovalent attachment in plastic microwells, arrays of synthetic carbohydrates linked to an aliphatic hydrocarbon. Galactose and seven di- to hexa-saccharides were derivatized to a 14-carbon aliphatic hydrocarbon and are reported to elicit binding signals in accord with predictions for the three plant lectins investigated.

43. • Fazio F, Bryan MC, Blixt O, Paulson JC, Wong CH: **Synthesis of sugar arrays in microtiter plate**. *J Am Chem Soc* 2002, **124**:14397-14402.

An alternative noncovalent attachment strategy for arraying carbohydrates in plastic microwells is described. The 14-carbon aliphatic chain selected in [42•] is first immobilized noncovalently in the wells; this is followed by the conjugation of azides of carbohydrates to the lipid. Galactose and eleven di- to tetra-saccharides, including those terminating with sialic acid, are thus immobilized and are reported to give the predicted binding signals with the plant lectins ricin B and *Sambucus nigra*. By monitoring for binding by the latter lectin, fucosylation of lipid-linked 3′-sialyl-*N*-acetyllactosmine within wells using α-1,3-fucosyltransferase and GDP-fucose is demonstrated. This is an example of microscale saccharide synthesis in microplates, with potential applications to high-throughput identification and kinetic analyses of substrates and inhibitors of glycosyltransferases (see also [37•]).

44. Leteux C, Stoll MS, Childs RA, Chai W, Vorozhaikina M, Feizi T: **Influence of oligosaccharide presentation on the interactions of carbohydrate sequence-specific antibodies and the selectins. Observations with biotinylated oligosaccharides**. *J Immunol Methods* 1999, **227**:109-119.

45. • Feizi T, Fukui S, Galustian C, Lawson AM, Chai W: **Progress toward deciphering the information content in the glycome.** In Proceedings of the 50th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics 2002 June 2–6; Orlando, Florida: abstractA021419.

See annotation to [41••].

46. Feizi T, Stoll MS, Yuen C-T, Chai W, Lawson AM: **Neoglycolipids: probes of oligosaccharide structure, antigenicity and function**. *Methods Enzymol* 1994, **230**:484-519.

47. Galustian C, Lubineau A, le Narvor C, Kiso M, Brown G, Feizi T: **L-selectin interactions with novel mono- and multisulfated Lewis^x sequences in comparison with the potent ligand 3′-sulfated Lewis^a**. *J Biol Chem* 1999, **274**:18213-18217.

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49. Kogelberg H, Solis D, Jimenez-Barbero J: **New structural insights into carbohydrate-protein interactions from NMR spectroscopy.** *Curr Opin Struct Biol* 2003, in press.

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51. Sears P, Wong CH: **Carbohydrate mimetics: a new strategy for tackling the problem of carbohydrate-mediated biological recognition**. *Angew Chem Int Ed Engl* 1999, **38**:2300-2324.

52. Bertozzi CR, Kiessling LL: **Chemical glycobiology**. *Science* 2001, **291**:2357-2364.

53. Schwarz M, Spector L, Gargir A, Shtevi A, Gortler M, Alstock RT, Dukler AA, Dotan N: **A new kind of carbohydrate array, its use for profiling anti-glycan antibodies, and the discovery of a novel human cellulose-binding antibody.** *Glycobiology* 2003, in press.