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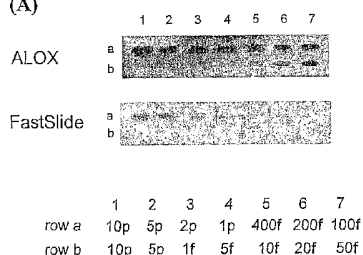
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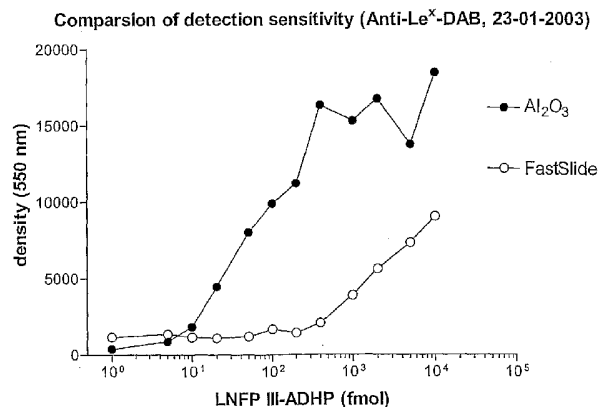
(54) Title: PRODUCTS AND METHODS

(A)



LNFP III-ADHP was used as an example and LNNT-ADHP (underlined) as negative control; p: pmol, f: fmol.

(B)



(57) Abstract: A support on which is immobilised a carbohydrate-containing molecule or molecules, wherein said support has multiple channels substantially perpendicular to the surface of the support onto which the carbohydrate-containing molecule or molecules is applied, and wherein the channels are open to the surface and have a diameter large enough to accommodate the carbohydrate-containing molecule. A support on which is immobilised a carbohydrate-containing molecule or molecules, wherein said support comprises a metal oxide. A method of determining whether a molecule interacts with a carbohydrate comprising: i) contacting a support according to the invention with the molecule; and ii) measuring whether the molecule binds to a carbohydrate-containing molecule or molecules on the support.



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Products and methods

The present invention relates to a support with immobilised carbohydrate-containing molecules, arrays of carbohydrate-containing molecules on said support material, and methods of using said supports with immobilised carbohydrate-containing molecules.

Carbohydrate chains are prominent components at the surface of mammalian cells occurring as *N*- and *O*-glycans on glycoproteins, glycosaminoglycan chains on proteoglycans, and oligosaccharides of glycolipids. Carbohydrate chains also occur on many secreted and extracellular glycoproteins. They range in length from two to more than two hundred monosaccharide residues, and they are almost unfathomably diverse. The term *glycome* has been coined for the repertoire of oligosaccharide structures in an organism.

Cellular glycoconjugates play important roles in many biological processes, including events of molecular recognition at fertilisation (Focarelli et al (2001) *Cells Tiss Org* 168, 76-81; Rosati et al (2000) *Int J Dev Biol* 44, 609-618) and processes of cell-cell recognition, adhesion, and cell activation throughout the development and maturation of a living organism (Feizi (1982) *Adv Exp Med Biol* 152, 167-177; Crocker and Feizi (1996) *Curr Opin Struc Biol* 6, 679-691; Feizi (2000) *Glycoconj J* 17, 553-565; Feizi (2000) *Immunol Rev* 173, 79-88). Abnormalities in the expression of complex carbohydrates are found in cancer (Hakomori (1985) *Cancer Res* 45, 2405-2414; Sell (1990) *Hum Pathol* 21, 1003-1019) retrovirus infection (Adachi et al (1988) *J Exp Med* 167, 323-331; Nakaishi et al (1988) *Cancer Res* 48, 1753-1758) and other diseases (Schachter and Jaeken (1999) *Biochem Biophys Acta* 1455, 179-192). Carbohydrate structures also play

critical roles in host-microorganism interactions. Many attachment sites or co-receptors for microbes are glycoconjugates (Karlsson et al (1992) APMIS Suppl 27, 71-83); their structural diversity and selectivity of host tissue expression contribute significantly to the tropism of microbial infections (Karlsson et al (1992) APMIS Suppl 27, 71-83; Feizi and Loveless (1996) Am J Respir Crit Care Med 154, S133-136).

Post genome, with the realization that the number of proteins encoded in the human genome is fewer than anticipated, there is heightened interest in carbohydrates and ways in which they diversify proteins and may modulate their activities and functions in health and disease. One challenge, for example, is to determine the repertoire of carbohydrate-binding proteins. There is an increasing number of receptors known to operate through binding to specific oligosaccharides. Among them are proteins that mediate critical processes such as protein folding and trafficking, and play key roles in cell-mediated and humoral mechanisms of inflammation and immunity (Helenius and Aebi (2001) Science 291, 2364 – 2369; Feizi (2000) Immunol Rev 173, 79-88; Crocker and Varki (2001) Trends Immunol 22, 337-342; Weis et al (1998) Immunol Rev 163, 19-34). Moreover, as mentioned above, a considerable number of pathogens have evolved to produce adhesive proteins that bind to specific carbohydrate sequences on host cells at the initial stages of infection (Karlsson (1998) Mol Microbiol 29, 1-11). This knowledge has served to stimulate ideas on carbohydrate-based therapeutics.

Developments in assignments of roles for carbohydrate sequences as ligands have lagged behind those for nucleic acid and protein sequences. This is in part because of the remarkable heterogeneities of carbohydrates, and the relatively small amounts that can be isolated. There are two other major factors. The first is that oligosaccharide ligands cannot be readily

cloned, being products of multiple glycosyltransferases. Second, the affinities of most carbohydrate-protein interactions are so low that di- or multivalence both of oligosaccharide and of the recognition protein is required for sensitive detection in binding experiments such as precipitation and radioimmunoassays, or ELISA-type experiments. Isolated free oligosaccharides can be examined only as inhibitors of such interactions. Hence there is a need for simple and readily accessible high-throughput analysis to be developed.

Microarrays have been reported to be one of the most frequently used screening approaches because large libraries of compounds can be quickly screened and only small quantities of material are required (Fodor et al (1991) *Science* 251, 767-773; Whitesides and Love (2001) *Sci Am* 285, 38-47; Cui et al (2001) *Science* 293, 1289-1292). This is an important consideration due to the low availability of some complex carbohydrates. However, few approaches have been developed, thus far, for the fabrication of carbohydrate microarrays.

Wang et al (2002) *Nature Biotech* 20, 275-281 report that nitrocellulose-coated glass slides can be used to immobilise microspots of carbohydrate polymers without covalent conjugation.

Houseman and Mrksich (2002) *Chemistry Biology* 9, 443-454 report that carbohydrate arrays can be prepared by the Diels-Alder mediated immobilisation of carbohydrate-cyclopentadiene conjugates to a gold surface.

Fazio et al (2002) *J Am Chem Soc* 124, 14397-14402 report the synthesis and in situ attachment of oligosaccharides to microtitre plates via a saturated hydrocarbon.

Fukui et al (2002) Nature Biotech 20, 1011-1017 report microarrays of oligosaccharides displayed as neoglycolipids on nitrocellulose.

Willats et al (2002) Proteomics 2, 1666-1671 report a polystyrene microarray slide surface that is capable of direct immobilisation of a variety of polysaccharides from plants.

A first aspect of the invention provides a support on which is immobilised a carbohydrate-containing molecule or molecules, wherein said support has multiple channels substantially perpendicular to the surface of the support onto which the carbohydrate-containing molecule or molecules is applied, and wherein the channels are open to the surface and have a diameter large enough to accommodate the carbohydrate-containing molecule.

The first aspect of the invention provides a carbohydrate-containing molecule presented in a manner that allows other molecules to bind the carbohydrate-containing molecule. As will be elaborated below, this may be used, amongst other purposes, to array carbohydrate-containing molecules, to identify molecule-carbohydrate interactions, or in the discovery of molecules that interfere with such binding.

As the channels are substantially perpendicular to the macroscopic surface of the support, it is considered that the carbohydrate-containing molecules enter a small macroscopic surface. However the actual surface area of the three-dimensional substrate may be approximately 500 times larger. Hence the detection of any molecule-carbohydrate interactions, while appearing to be localised to the small macroscopic surface also includes the surface area of the channels. There may be a better 'signal to noise' ratio than when the

carbohydrate-containing molecules can spread out, as is considered to be the case with support materials used in the art.

A further advantage of the first aspect of the invention over other means of displaying carbohydrates may be that much smaller quantities of carbohydrate-containing molecules have to be immobilised on the support material for use in detecting molecule-carbohydrate interactions. This may be in the order of 10 to 100 times less than that used for other carbohydrate containing or displaying support materials. This may be due to the carbohydrate-containing molecules being more efficiently immobilised in a small macroscopic area, as set out above.

Such an advantage allows for carbohydrates that are available in extremely small amounts from natural sources to be immobilised. For example, using the present invention an array of carbohydrate-containing molecules can be prepared from manageable numbers of whole cells, for example 10^{10} , or organs.

While not wishing to be bound to a particular theory, it is thought that the carbohydrate-containing molecules can be immobilised within the channels in the support of the invention, ie on the walls of the channels. This is considered to give a higher retention of the carbohydrate-containing molecule on the material than is the case with other support materials which can be used to display carbohydrates.

By 'support' we mean a material (which may be a composite) on which to immobilise the carbohydrate-containing molecule or molecules that can resist change in size or shape during normal use. Examples of support materials are given below. Composite materials may comprise a component material which can act to provide solidity to the support as well as a

component material having multiple channels substantially perpendicular to the surface of the support. For example the support may be a glass slide coated with a component material having multiple channels substantially perpendicular to the surface of the support.

By 'carbohydrate-containing molecule' we include all molecules that are carbohydrates or contain carbohydrate. By 'carbohydrate' we include sugar, saccharide, or glycan, as well as monosaccharides, oligosaccharides, and polysaccharides and derivatives of these compounds as discussed further below. A carbohydrate-containing molecule may be, for example, a glycoprotein, a glycolipid, or a proteoglycan. Further types of carbohydrate-containing molecule are discussed below.

Preferably, the support of the first aspect of the invention has channels of between 0.01 and 5 μm in diameter, preferably 0.05 to 2 μm in diameter, preferably 0.1 to 0.5 μm in diameter. Most preferably, the support material has channels of 0.2 μm in diameter.

While not wishing to be bound to a particular theory, it is thought that the preferred range of channel diameters allows the carbohydrate-containing molecule to enter into and be immobilised within the channel. Once immobilised the carbohydrate-containing molecule can be accessed by other molecules which may interact with the carbohydrate.

Preferably, the support of the first aspect of the invention has channels at a density of 1 to 100 channels / μm^2 , preferably 10 to 50 channels / μm^2 . More preferably the support of the first aspect of the invention has channels at a density of 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45 channels / μm^2 . More preferably, the support has

channels at a density of between 32 and 38 channels / μm^2 , for example 36 channels / μm^2 .

While not wishing to be bound to a particular theory, it is thought that the preferred range of channel densities allows carbohydrate-containing molecules to be immobilised within channels at a high density. This has the effect of localising the carbohydrate-containing molecules in a small macroscopic surface area. When used in an array, the support has a high density of locations in a given area at which carbohydrate-containing molecules can be immobilised.

As mentioned above, the support of the first aspect of the invention has multiple channels, of a diameter large enough to accommodate a carbohydrate-containing molecule, substantially perpendicular to the surface of the support onto which the carbohydrate-containing molecule is applied. The channels may also be large enough to accommodate a potential carbohydrate-binding molecule eg a protein or polypeptide, such that the potential carbohydrate-binding molecule can access the carbohydrate-containing molecule.

Thus, the support may have multiple channels of a diameter large enough to accommodate both a carbohydrate-containing molecule and a potential carbohydrate-binding molecule.

In a further embodiment of this aspect of the invention such a support is or comprises a metal oxide. A further embodiment of this aspect of the invention such a support is or comprises an electrochemically manufactured metal oxide membrane. In a further embodiment of this aspect of the invention such a metal oxide is or comprises aluminium oxide. Other metal oxides which may be suitable for the present invention include oxides of

tantalum, titanium as well as alloys of two or more metals and doped metals and alloys. Examples of such materials may be obtained from Merck KGaA, Darmstadt, Germany.

US Patent 6,225,131 to van Damme *et al* describes suitable support materials of the first aspect of the invention. The aluminium oxide support material described in this document can be obtained from PamGene B.V., PO Box 1345, 5200 BJ's-Hertogenbosch, The Netherlands.

The metal oxides, for example aluminium oxides described in US Patent 6,225,131 to van Damme *et al*, are considered to have through-going channels that are hydrophilic in comparison to the surface of the material. Thus a hydrophilic liquid preferably enters the channels instead of spreading over the surface of the material. Therefore, the metal oxide, for example aluminium oxide, material may accommodate high densities of locations comprising carbohydrate-containing molecules.

The molecules may be immobilised via non-covalent interactions: hydrogen bonding and ionic bonding and van der Waals interaction.

A second aspect of the invention provides a support on which is immobilised a carbohydrate-containing molecule or molecules, wherein said support comprises a metal oxide.

The second aspect of the invention also provides a carbohydrate-containing molecule presented in a manner that allows other molecules to bind the carbohydrate-containing molecule. As will be elaborated below, this may be used, amongst other purposes, to array carbohydrate-containing molecules, to identify molecule-carbohydrate interactions, or in the discovery of molecules that interfere with such binding.

A metal oxide is considered to provide suitable chemical properties for immobilisation of carbohydrate-containing molecules. Examples of metal oxides that may be suitable for this aspect of the invention include titanium oxide, tantalum oxide and aluminium oxide. Examples of such materials may be obtained from Merck KGaA, Darmstadt, Germany.

In a further embodiment of second aspect of the invention such a support is or comprises a metal oxide gel. A metal oxide gel is considered to provide a large surface area within a given macroscopic area to aid immobilisation of the carbohydrate-containing molecules.

In a further embodiment of this aspect of the invention such a metal oxide is aluminium oxide.

In a preferred embodiment of the first and second aspects of the invention the immobilised carbohydrate-containing molecule or molecules are arrayed on the support. By 'arrayed on' is included the arrayed molecules being immobilised within the channels running perpendicular to the macroscopic surface of the support materials of the first aspect of the invention.

The samples of carbohydrate-containing molecules can be placed at a high density. This is because the support materials may introduce the carbohydrate-containing molecules from a sample not only into a small macroscopic surface area but also into the substrate below. Therefore, it is considered possible to prepare a single array (for example occupying an area similar to that of a standard microscope slide) having more samples and hence potentially a much broader range of carbohydrate molecules than is possible with, for example, a nitrocellulose support.

Furthermore, both the first and second aspects of the invention may allow for a wide range of types of carbohydrate-containing molecules to be immobilised on the support. It has the potential to cover the entire range of carbohydrates present in nature ("glycomes") and in addition chemically synthesized carbohydrates. Both aspects of the invention may allow a similar proportion of applied carbohydrate-containing molecules of different types to be immobilised.

In addition, because a much smaller quantity of carbohydrate-containing molecules can be used for detecting molecule-carbohydrate interactions, arrays can be prepared using in the order of 10 to 100 times fewer carbohydrate-containing molecules of a given type than are necessary for other carbohydrate containing or displaying support materials. For example, an array comprising a support material of the invention can be prepared using 0.5, 1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 2, or 2.5 fmol of carbohydrate-containing molecules at each location on the array.

By 'arrayed on the support' we mean that one or more carbohydrate-containing molecules are immobilised as an organised arrangement or pattern at two or more locations on the support. The type of sample at each location is known. The term 'array' will be well known to those skilled in the art. See, for example, EP 0 804 731. Microarrays are also considered to be an embodiment of the first and second aspects of the invention. A microarray may typically have sample locations separated by a distance of 50-200 microns or less and immobilised sample in the nano to micromolar range or nano to picogram range. See, for example, EP 0 804 731.

Typically, an array will have at least 4, 8, 16, 24, 48, 96 or several hundred or thousand sample locations.

Arrays of other biomolecules are well known in the art, for example protein and nucleic acid arrays.

Biological chips or arrays have immobilised molecules arranged in arrays, with each immobilised molecule assigned a specific location. Biological chips have been produced in which each location has a scale of, for example, ten microns. The chips can be used to determine whether target molecules interact with any of the immobilised molecules on the chip. After exposing the array to target molecules under selected test conditions, scanning devices can examine each location in the array and determine whether a target molecule has interacted with the immobilised molecule or molecules at that location.

Biological chips or arrays are useful in a variety of screening techniques for obtaining information about either the immobilised molecules or the target molecules. For example, a library of immobilised carbohydrate-containing molecules can be used in screening for drugs. Or the carbohydrate-containing molecules can be exposed to a receptor, and those molecules that bind to the receptor can be identified. Molecules that interfere with such binding can be identified.

It is important to consider arrangements in which the carbohydrate-containing molecules can be arrayed. There are least two possible variables: the composition of the carbohydrate-containing molecules at each location of the array, and the composition of the carbohydrate-containing molecules at different locations of the array.

Therefore, one array included in this embodiment of the invention is where the same carbohydrate-containing molecule or molecules (for example as a complex sample) are immobilised on the support at one or more locations;

each of these locations containing the same carbohydrate-containing molecule or mixture of molecules. Hence this array includes an organised arrangement of samples of the same carbohydrate-containing molecule (or same mixture of molecules).

The location may contain the same quantities of the carbohydrate-containing molecule or mixture of molecules or may contain different quantities.

A further array included in this embodiment of the invention is where locations of the array differ in the carbohydrate-containing molecule or mixture of molecules that they contain. Hence this array includes an organised arrangement of samples of different carbohydrate-containing molecules or different mixtures of carbohydrate-containing molecules.

For example, the array may include samples of complex mixtures of carbohydrate-containing molecules from different cell types or tissues; or may include samples that correspond to fractions of such complex mixtures; or may include samples that correspond to single molecular species isolated from such fractions or prepared synthetically. Each type of sample may be present in more than one location (optionally at different concentrations) and one array may include examples of each such type of sample.

Non-limiting arrangements in which carbohydrate-containing molecules may be arrayed are shown in Figure 9. Here a support according to the first or second aspects of the invention is shown on which is immobilised an array of carbohydrate-containing molecules. The molecules present at location A1 may be identical to the molecules present at location A2, but A1 may have 10 times more molecules than at A2. Alternatively the molecules present at A1 may differ to those at A2, while B1 may be a repeat

location to that of A1 for the purposes of a control, ie A1 and B1 contain the same molecules. Other arrangements will be appreciated by those skilled in the art.

As will be set out below, the carbohydrate-containing molecules can be prepared from a broad range of sources. Therefore the arrays included in this embodiment of the first and second aspects of the invention can have immobilised carbohydrate-containing molecule or mixture of molecules prepared from the same source, or different sources.

As mentioned above, the support materials used in the invention are considered to allow a wide range of types of carbohydrate to be immobilised.

In an embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules is a polysaccharide. By 'polysaccharide' we mean carbohydrate composed of monosaccharides, generally greater than twenty monosaccharide units in length, as will be appreciated by those skilled in the art.

Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a polysaccharide. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the immobilised polysaccharide is arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules is a glycolipid. By 'glycolipid' we mean a molecule containing a saccharide linked to a lipid

aglycone, for example glycosphingolipids or lipopolysaccharides, as will be appreciated by a person skilled in the art.

Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a glycolipid. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the immobilised glycolipid is arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules is a glycosaminoglycan. Such molecules are polysaccharide side-chains of proteoglycans or free complex polysaccharides composed of disaccharide repeating units, each composed of a hexosamine and a hexose or a hexuronic acid (see heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate and hyaluronan).

Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a glycosaminoglycan or an oligosaccharide fragment of a glycosaminoglycan. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the immobilised glycosaminoglycan or an oligosaccharide fragment of a glycosaminoglycan is arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules is a glycoprotein. Such molecules are proteins with one or more covalently linked glycans, as will be well known to those skilled in the art.

Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a glycoprotein. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the immobilised glycoprotein is arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules is a reducing end-tagged molecule.

By 'reducing end-tagged molecule' we mean carbohydrate conjugated to a tag at its reducing end, which may assist in immobilising the carbohydrate to a support. Examples of suitable reducing end-tagged molecules include neoglycolipids, glycolipids, aminopyridine and phosphatidylethanolamine. In a preferred embodiment of the first and second aspects of the invention the reducing end-tagged molecule is a neoglycolipid or glycolipid.

Neoglycolipids are monosaccharides or oligosaccharides chemically conjugated to lipid molecules (Tang et al (1985) *Biochem Biophys Res Comm* 132, 474-480; Feizi et al (1994) *Methods Enzymol* 230, 484-519; WO 87/02777). Such lipid molecules include phosphatidylethanolamine-type aminolipids.

Tagging of each monosaccharide or oligosaccharide with a hydrophobic lipid tail confers amphipathic property such that the neoglycolipids can be immobilised (on matrices, such as the support materials of use in the first and second aspects of the invention as described above) with the carbohydrates considered to be displayed in a cluster.

The lipid molecules which can be used as tags for neoglycolipids include dipalmitoyl phosphatidylethanolamine (DPPE), 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE) and *N*-aminoacetyl-*N*-(9-anthracenylmethyl)-1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (ADHP). The tag may have a 10, 15, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55 or 60 carbon atom length. It is preferred that the neoglycolipid of this embodiment of the invention comprises a tag of between 24 to 50 carbon atom length.

The reducing end-tagged molecule may have a lipid tag of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35 or 40 carbon atom length. It is preferred that the reducing end-tagged molecule comprises a tag of between 5 to 25 carbon atom length, with an aliphatic or aromatic hydrocarbon backbone.

Also preferred is an embodiment of the first and second aspects of the invention where the tag or the resulting neoglycolipid has a chromophore. For example, aminopyridine has a chromophore.

Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a reducing end-tagged molecule or neoglycolipid. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the immobilised reducing end-tagged molecule or neoglycolipid is arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the reducing end-tagged molecule or neoglycolipid contains an oligosaccharide. By 'oligosaccharide' we mean a linear or branched chain of

monosaccharides attached to one another via glycosidic linkages. The number of monosaccharide units can vary; the term polysaccharide is usually reserved for large glycans with more than 20 monosaccharide units. In general an oligosaccharide has less than 20 monosaccharide units.

In a further embodiment of the first and second aspects of the invention the reducing end-tagged molecule or neoglycolipid contains a monosaccharide. By 'monosaccharide' we mean carbohydrate that cannot be hydrolyzed into a simpler carbohydrate, ie they are the building block of oligosaccharides and polysaccharides. Simple monosaccharides are polyhydroxyaldehydes or polyhydroxyketones with three or more carbon atoms.

In a further embodiment of the first and second aspects of the invention the reducing end-tagged molecule or neoglycolipid contains a *N*-glycan. This carbohydrate is glycan covalently linked to an asparagine residue of a polypeptide chain in the consensus sequence: -Asn-X-Ser/Thr.

In a further embodiment of the first and second aspects of the invention the reducing end-tagged molecule or neoglycolipid contains an *O*-glycan. This carbohydrate is a glycan glycosidically linked to the hydroxyl group of the amino acids eg serine and threonine. Where the oligosaccharide is *O*-glycan, in a further embodiment of this aspect of the invention the *O*-glycan terminates, for example, in *N*-acetylgalactosamine, *N*-acetylgalactosaminitol, mannose or mannitol.

In a further embodiment of the first and second aspects of the invention the reducing end-tagged molecule or neoglycolipid contains a fragment of glycosaminoglycan.

The carbohydrate of the reducing end-tagged molecule or neoglycolipid of the first and second aspects of the invention can be prepared from a broad range of sources. The methods used for isolating oligosaccharide or monosaccharides and the subsequent preparation of neoglycolipids is outlined in Fukui et al (2002) *Nature Biotech* 20, 1011-1017 and WO 87/02777, both incorporated herein by reference.

Hence a further embodiment of the first and second aspects of the invention is where the reducing end-tagged molecule or neoglycolipid has an oligosaccharide or monosaccharide derived from one or more carbohydrate sources selected from glycoproteins, glycolipids, GPI-linked glycans, proteoglycans/glycosaminoglycans and polysaccharides or is synthesised chemically.

In a further embodiment of the first and second aspects of the invention the oligosaccharide or monosaccharide is a reducing sugar. In a further embodiment of the first and second aspects of the invention the oligosaccharide or monosaccharide is a reduced sugar.

Where the oligosaccharide or monosaccharide is a reduced sugar, a further embodiment of the first and second aspects of the invention is where the oligosaccharide or monosaccharide is tagged at the reduced terminal after a mild oxidation procedure as described in WO 87/02777 and Stoll et al (1990) *Eur J Biochem* 189, 499-507.

As noted above, the support materials of the first and second aspects invention can have one or more samples of immobilised carbohydrate-containing molecules in which the carbohydrates within a sample are the same. Alternatively, the carbohydrate-containing molecules within a sample may have carbohydrates which are different.

Thus, in a further embodiment of the first and second aspects of the invention the immobilised carbohydrate-containing molecule or molecules comprise a homogeneous sample of carbohydrate. Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised one or more samples of carbohydrate-containing molecules which comprise a homogeneous sample of carbohydrate. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the carbohydrate-containing molecules are arrayed or non-arrayed. Therefore, each sample of carbohydrate-containing molecules contains a single molecular species of carbohydrate.

Such an embodiment of the invention can be used, for example, to detect whether a specific carbohydrate-interacting molecule is present in a test sample. This will be further detailed below. It can also be used to determine the binding specificity of a specific carbohydrate-interacting molecule.

In a further embodiment of the first and second aspects the invention the carbohydrate-containing molecule or molecules comprise a heterogeneous sample of carbohydrate. Hence an embodiment of the first and second aspects of the invention can be a support on which is immobilised one or more samples of carbohydrate-containing molecules which comprise a heterogeneous sample of carbohydrate-containing molecules. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the carbohydrate-containing molecules are arrayed or non-arrayed. Therefore, each sample of carbohydrate-containing molecules is a complex mixture of different molecular species of carbohydrate-containing molecules.

Such an embodiment of the invention can be used, for example, to detect which general type or specific carbohydrate a carbohydrate-interacting molecule can interact with. This will be further detailed below.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules comprises a carbohydrate derived from a microbe. For example, the carbohydrate may be derived from a bacterium, a fungus, or a virus. Such embodiments can be of particular use, for example, in identifying molecules which interact with carbohydrates from microbes. Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a carbohydrate-containing molecule or molecules which comprises a carbohydrate derived from a microbe. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the carbohydrate-containing molecules are arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules comprises a carbohydrate derived from a specific cell type. For example, the carbohydrate may be obtained from cancer cells. Such embodiments can be of particular use, for example, in identifying molecules which interact with carbohydrates from cancerous cells. Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a carbohydrate-containing molecule or molecules which comprises a carbohydrate derived from a specific cell type. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the carbohydrate-containing molecules are arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules comprises a carbohydrate derived from a specific tissue or organ. For example, the carbohydrate may be obtained from brain, spleen or muscle. Such embodiments can be of particular use, for example, in identifying molecules which interact with carbohydrates from the tissues. Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a carbohydrate-containing molecule or molecules which comprises a carbohydrate derived from a specific tissue or organ. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the carbohydrate-containing molecules are arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules comprises a carbohydrate derived from a cell type or tissue from an animal, for example a mouse, rat, rabbit, dog, cat, chimpanzee or human. Preferably, said animal is a human. Such embodiments can be of particular use, for example, in identifying molecules which interact with carbohydrates from a cell type or tissue or organ of a human. Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a carbohydrate-containing molecule or molecules which comprises a carbohydrate derived from an animal, for example a human. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the carbohydrate-containing molecules are arrayed or non-arrayed.

In a further embodiment of the first and second aspects of the invention the carbohydrate-containing molecule or molecules comprises a carbohydrate derived from a cell type or tissue or organ from a plant. For example, the

carbohydrate may be obtained from *Arabidopsis thaliana*, maize, wheat or rice. Such embodiments can be of particular use, for example, in identifying molecules which interact with carbohydrates from a cell type or tissue of a plant. Hence an embodiment of the first and second aspects of the invention is a support on which is immobilised a carbohydrate-containing molecule or molecules which comprises a carbohydrate derived from a cell type or tissue from a plant. By this we include all embodiments of the support as set out in the first or second aspects of the invention, including where the carbohydrate-containing molecules are arrayed or non-arrayed.

As discussed above, the first and second aspects of the invention comprises a carbohydrate-containing molecule or molecules immobilised on a support. However, it is generally necessary to first solubilise the carbohydrate-containing molecules to allow them to be immobilised on the support. Some carbohydrate-containing molecules are water soluble and hence can be applied to the support when dissolved in water. Examples of such carbohydrate-containing molecules include monosaccharides, oligosaccharides, polysaccharides, glycosaminoglycans (GAGs), proteoglycans and glycoproteins.

However, some carbohydrate-containing molecules are poorly soluble in water. Examples of such carbohydrate-containing molecules include glycolipids and neoglycolipids. Hence it may be necessary or desirable to use other solvents to dissolve or suspend these carbohydrate-containing molecules so they can be immobilised on a support of the first or second aspects of the invention.

Currently, the solvents used to solubilise neoglycolipids and glycolipids are organic-based solvent mixtures usually containing chloroform (see, for example, Fukui et al (2002) Nat Biotech 20, 1011-1017). Chloroform is

often an essential component of the solvent but it can be problematic as it is highly volatile. For example, the chloroform/methanol/water 25:25:8 by volume solvent used by Fukui et al (2002) Nat Biotech 20, 1011-1017 cannot be used in some modern arrays of either contact or non-contact types.

We have developed alternative aqueous/low volatility solvents that can be used to solubilise neoglycolipids and glycolipids. Such a solvent may be of use in, for example, immobilising neoglycolipids or glycolipids on a support of the first or second aspects of the invention. The solvents are comprised of aqueous/aliphatic alcohol mixtures.

Hence a further aspect of the invention is a method of preparing a support according to the first or second aspects of the invention wherein the carbohydrate-containing molecule or molecules is immobilised on the support while solubilised in a solvent comprising an aqueous/aliphatic alcohol mixture. Preferably, the carbohydrate-containing molecule is a reducing end-tagged molecule, preferably a neoglycolipid or a glycolipid.

A further embodiment of this aspect of the invention is where the solvent includes an aliphatic alcohol selected from a list comprising: propanol (propan-1-ol), iso-propanol (propan-2-ol), n-butanol (butan-1-ol), iso-butanol (butan-2-ol), and t-butanol (2-methylpropan-2-ol). Preferably, the aliphatic alcohol constitutes between 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25 or 30% of the solvent by volume. More preferably, the aliphatic alcohol constitutes between 8 to 15% (v/v) of the solvent.

While not wishing to be bound to a particular theory, it is thought that the carbohydrate-containing molecule or molecules are immobilised on a support according to the first or second aspects of the invention via a non-

covalent interaction, such as hydrogen bonding, ionic or van der Waals interaction.

A further aspect of the invention is a method for detecting a molecule in a test sample comprising:

- i) contacting a support according to the first or second aspects of the invention with the test sample; and,
- ii) detecting the binding of any molecules in the test sample to the carbohydrate-containing molecule or molecules.

We include all embodiments of the support as set out in the first and second aspects of the invention, including where the immobilised carbohydrate-containing molecules are arrayed or non-arrayed.

By 'a test sample' we include a sample of a body fluid such as blood, serum, plasma, urine, cerebrospinal fluid, pleural fluid and semen.

The binding of a molecule in the test sample to the carbohydrate-containing molecule can be measured using, for example, colorimetric or fluorescence detection systems, or other labelling methods, or other methods that do not require labelling.

Such a method may be of particular use in, for example, detecting the presence of a specific protein in serum, eg antibody, by its binding to an immobilised carbohydrate-containing molecule or molecules. In this way it could be determined whether a patient has contracted a disease caused by a certain microbe or parasite. An example of how such a method could be used is disclosed in Example 3.

A further aspect of the invention is a method of determining whether a molecule interacts with a carbohydrate comprising:

- i) contacting a support according to the first or second aspects of the invention with the molecule; and
- ii) measuring whether the molecule binds to a carbohydrate-containing molecule or molecules on the support.

An embodiment of this aspect of the invention is where the carbohydrate-containing molecules are arrayed on the support.

The binding of a molecule to the carbohydrate-containing molecule or molecules can be measured using, for example, colorimetric or fluorescence detection systems, or other labelling methods, or other methods that do not require labelling.

Such a method may be of particular use in, for example, identifying whether a molecule thought to be capable of interacting with a carbohydrate can actually do so, or to identify whether a molecule unexpectedly has the capability of interacting with a carbohydrate.

Also included in this method is where the immobilised samples of carbohydrate-containing molecules include complex mixtures of carbohydrate-containing molecules, for example forming an array of heterogeneous samples of carbohydrate molecules. In this method a molecule can be assayed to determine whether it interacts with carbohydrates present in, for example, a human cell or tissue type. Such a method may be of use in screening possible therapeutic molecules to

identify those molecules which bind to carbohydrate-containing molecules. An example of how such a method could be used is disclosed in Example 4.

A further aspect of the invention is a method of determining the kinetics of interaction between a molecule and a carbohydrate comprising:

- i) contacting a support according to the first or second aspects of the invention with a molecule; and
- ii) measuring the kinetics of interaction between the molecule and the carbohydrate-containing molecule or molecules on the support.

We include all embodiments of the support as set out in the first and second aspects of the invention, including where the immobilised carbohydrate-containing molecules are arrayed or non-arrayed.

Therefore, an embodiment of this aspect of the invention is where the carbohydrate-containing molecules are arrayed on the support.

The kinetics of interaction of a molecule to any of the carbohydrate-containing molecules can be measured by real time changes in, for example, colorimetric or fluorescent signals.

Such a method may be of particular use in, for example, determining whether a molecule is able to interact with a specific carbohydrate with a higher degree of binding than a different molecule to the same carbohydrate. This may be of use in identifying a therapeutic molecule which can bind with a high affinity to carbohydrate-containing molecules. An example of how such a method could be used is disclosed in Example 5.

A preferred embodiment of this aspect of the invention is where the support used is of the first aspect of the invention and all embodiments of the first aspect of the invention.

A further aspect of the invention is a method of identifying a carbohydrate-binding molecule in a heterogeneous sample of molecules comprising:

- i) contacting a support according to the first or second aspects of the invention with a heterogeneous sample of molecules; and
- ii) identifying a molecule or molecules which interact with a carbohydrate-containing molecule or molecules on the support.

We include all embodiments of the support as set out in the first and second aspects of the invention, including where the immobilised carbohydrate-containing molecules are arrayed or non-arrayed.

Therefore, an embodiment of this aspect of the invention is where the carbohydrate-containing molecules are arrayed on the support.

The interaction of a molecule to the carbohydrate-containing molecule can be measured by colorimetric or fluorescence detection systems, or other labelling methods, or other methods that do not require labelling.

Such a method may be of particular use in, for example, identifying whether any molecules present in a test sample are capable of interacting with the carbohydrate-containing molecule or molecules immobilised on the support. Also included in this method is where the support is an array of heterogeneous samples of carbohydrate-containing molecules. In this method a heterogeneous population of molecules can be assayed to identify

those molecules that interact with carbohydrates present in, for example, a human cell or tissue type. Such a method may be of use in identifying possible therapeutic molecules. An example of how such methods may be used is disclosed in Example 6.

A further aspect of the invention is a method of identifying a carbohydrate bound by a molecule (optionally from a heterogeneous population of molecules) comprising:

- i) contacting a support according to the first or second aspects of the invention, with the molecule; and
- ii) identifying the carbohydrate-containing molecule or molecules on the support to which the molecule binds.

We include all embodiments of the support as set out in the first and second aspects of the invention where the immobilised carbohydrate-containing molecules are arrayed on the support.

Preferably the support has more than one carbohydrate-containing molecule immobilised thereof, preferably in the form of an array.

The interaction of a molecule to any of the carbohydrate-containing molecule or molecules can be measured by colorimetric or fluorescence detection systems, or other labelling methods, or other methods that do not require labelling.

Such a method may be of particular use in, for example, identifying which carbohydrate-containing molecule or molecules (optionally in a heterogeneous population of such molecules) are able to bind to a test

sample or a molecule. For example, if it is known that a specific molecule interacts with a carbohydrate-containing molecule in a cell then the method can be used to identify the specific carbohydrate-containing molecule to which the molecule binds. An example of how such a method could be used is disclosed in Example 7.

In a further embodiment of this aspect of the invention the method further comprises a deconvolution process. The purpose of a deconvolution process is to identify specific carbohydrate-containing molecules, to which a molecule binds, in a complex mixture of carbohydrate-containing molecules. One such method involves: isolating a complex mixture of carbohydrate-containing molecules to which a test sample or molecule binds, making a 'daughter' array of the complex mixture of carbohydrate-containing molecules separated into individual or a restricted number of molecules, binding the test sample or molecule to the 'daughter' array, and identifying the carbohydrate-containing molecule or molecules to which the test sample or molecule binds, using mass spectrometry preceded as necessary by thin-layer or multi-dimensional chromatographies and chromatogram binding.

A further aspect of the invention is a method of separating specific cells from a heterogeneous population of cells comprising:

- i) providing a support according to the first or second aspects of the invention, wherein the carbohydrate-containing molecule or molecules is able to interact with specific cells;
 - ii) contacting the support with a heterogeneous population of cells;
- and

iii) separating those cells that bind to the carbohydrate-containing molecule or molecules from those cells that do not bind to the carbohydrate-containing molecule or molecules on the support.

We include all embodiments of the support as set out in the first and second aspects of the invention, including where the immobilised carbohydrate-containing molecules are arrayed or non-arrayed.

Such a method may be of particular use in, for example, identifying cells that have on their surface a molecule which binds to the carbohydrate-containing molecule or molecules on the support. For example, the method may be of use in panning experiments to isolate cells having specific molecules on their surface, as will be appreciated by those skilled in the art. The method may also be of use in identifying microbes from the urine of a patient having, or suspected of having, a urinary infection caused by a bacterium. An example of how such methods can be used is disclosed in Example 8.

A further aspect of the invention is a method of determining whether a test molecule interferes with the binding of a molecule or cell to a carbohydrate-containing molecule comprising:

- i) providing a support according to the first or second aspects of the invention, wherein the carbohydrate-containing molecule or molecules is bound by a molecule or cell;
- ii) contacting the support with a test molecule; and

iii) identifying whether the test molecule interferes with the binding of a molecule or cell to the carbohydrate-containing molecule or molecules on the support.

We include all embodiments of the support as set out in the first and second aspects of the invention, including where the immobilised carbohydrate-containing molecules are arrayed or non-arrayed, or homogeneous or heterogeneous.

The identification of whether the molecule interferes with the binding of a molecule or cell to the carbohydrate-containing molecule can be measured by, for example, detecting changes in the quantity or type of molecule bound to the carbohydrate-containing molecule or molecules on the support.

Such a method may be of particular use to, for example, identify whether a molecule, for example a small drug, carbohydrate or carbohydrate-mimic, can interfere with the binding of a molecule to a carbohydrate. This could be the basis of a competition/inhibition assay screen as would be appreciated by a person skilled in the art. An example of how such a method could be used is disclosed in Example 9.

In a further embodiment of this aspect of the invention the test molecule is part of a heterogeneous population of molecules.

Such a method may be of particular use, for example, to identify which molecules in a heterogeneous population of molecules can interfere with the binding of another molecule to a carbohydrate. An example of how such a method could be used is disclosed in Example 9.

In a further embodiment of the methods of the invention, the molecule that may interact with a carbohydrate-containing molecule, or a test molecule that may interfere with the binding of a molecule to a carbohydrate-containing molecule, may be a polypeptide, for example, an antibody, enzyme, receptor, lectin or glycoprotein. The molecule may also be a peptidomimetic, nucleic acid, carbohydrate, lipid, glycolipid, hormone, microbial antigen or a glycomimic. The molecule may also be a therapeutic molecule, for example, a therapeutic molecule smaller than 500 daltons, a prophylactic, a vaccine, or an immunomodulator.

The term "peptidomimetic" refers to a compound that mimics the conformation and desirable features of a particular peptide as a therapeutic agent, but that avoids the undesirable features. For example, morphine is a compound which can be orally administered, and which is a peptidomimetic of the peptide endorphin. There are a number of different approaches to the design and synthesis of peptidomimetics, such as those discussed in Sherman and Spatola, *J. Am. Chem. Soc.*, 112: 433 (1990), Meziere *et al* (1997) *J. Immunol.* **159** 3230-3237, Veber *et al*, *Proc. Natl. Acad. Sci. USA*, 75:2636 (1978), Thursell *et al*, *Biochem. Biophys. Res. Comm.*, 111:166 (1983) and D. H. Rich in *Protease Inhibitors*, Barrett and Selveson, eds., Elsevier (1986), all incorporated herein by reference.

A further aspect of the invention is a molecule as identified by any of the methods of the invention disclosed above.

A further aspect of the invention is a carbohydrate as identified by any of the methods of the invention disclosed above.

As discussed above we have developed alternative aqueous/low volatility solvents that can be used to solubilise neoglycolipids and glycolipids. The

solvents are comprised of aqueous/aliphatic alcohol mixtures. Such solvents can be used solubilise neoglycolipids and glycolipids prior to immobilising the molecules on a support material.

Hence a further aspect of the invention is the use of a solvent comprising an aliphatic alcohol for solubilising a carbohydrate-containing molecule or molecules in the preparation of a support according to the first and second aspects of the invention. Preferably, the carbohydrate-containing molecule is a reducing end-tagged molecule, preferably a neoglycolipid or a glycolipid.

In this aspect of the invention a carbohydrate-containing molecule or molecules is first solubilised using a solvent comprising an aliphatic alcohol. The solubilised carbohydrate-containing molecule or molecules is then immobilised on the support material.

An advantage of using such a solvent over existing solvents for is that a solvent comprising an aliphatic alcohol is less volatile and can be used in some modern arrayers of either contact or non-contact types.

A further embodiment of this aspect of the invention is where the solvent used has an aliphatic alcohol selected from a list comprising: propanol (propan-1-ol), iso-propanol (propan-2-ol), n-butanol (butan-1-ol), iso-butanol (butan-2-ol), and t-butanol (2-methylpropan-2-ol). Preferably, the aliphatic alcohol constitutes between 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25 or 30% of the solvent by volume. More preferably, the aliphatic alcohol constitutes between 8 to 15% (v/v) of the solvent.

Any publications referred to herein are hereby incorporated by reference.

The invention will now be described in more detail by reference to the following non-limiting Figures and Examples.

Figure 1: Comparison of detection sensitivity of NGLs on ALOX and nitrocellulose-coated FastSlide by anti-Le^x antibody.

In (A) different amounts (1 fmol to 10 pmol) of fluorescent NGLs were applied as 2 mm bands on aluminium oxide membrane (ALOX) and nitrocellulose coated FastSlide. The Le^x pentasaccharide LNFP III-ADHP was used as an example to demonstrate the detection sensitivity by an anti-Le^x antibody; a tetrasaccharide LNNT-ADHP (positions 1b and 2b) was used as the negative control. Binding was revealed by the development of DAB colour reaction. In (B) the binding intensity revealed by the DAB colour of each bands was scanned at 550 nm, recorded and plotted for direct comparison.

Figure 2: Anti-chondroitin sulphate (CS) antibody binding to CSC polysaccharide and CSC 18mer-DHPE neoglycolipid.

Different amounts of CSC polysaccharide (5 to 500 ng) and its oligosaccharide derived NGL, CSC 18mer-DHPE (0.05 to 5 pmol), were applied as 2 mm bands on aluminium oxide membrane (ALOX) and nitrocellulose coated FastSlide. Binding was revealed by the development of DAB colour reaction.

Figure 3: Protein TSG-6 binding to hyaluronic acid (HA) and HA 8mer-DHPE neoglycolipid.

Different amounts of HA polysaccharide (5 to 500 ng) and its oligosaccharide derived NGL, HA 8mer-DHPE (0.05 to 5 pmol), were applied as 2 mm bands on aluminium oxide membrane (ALOX) and nitrocellulose coated FastSlide. Binding was revealed by the development of DAB colour reaction.

Figure 4: Binding of a biotinylated HA-binding protein to different preparations of HA.

Different HA polysaccharide preparations (0.5 to 500 ng) were applied as 2 mm bands on aluminium oxide membrane (ALOX) and nitrocellulose coated FastSlide. HA-BVH: from bovine vitreous humor; HA-S: from Streptococcus; HA-S350: HA-S with 350 kDa; HA-S220: HA-S of 220 kDa. Binding was revealed by the development of DAB colour reaction.

Figure 5: Anti-thrombin III binding to heparin/heparan sulphate polysaccharides.

Different amounts of heparin, heparan sulphate and de-N-acetylated heparin polysaccharide (5 to 500 ng), and chondroitin sulphates A and C together with dextran sulphate (500 ng each) were applied as 2 mm bands on aluminium oxide membrane (ALOX) and nitrocellulose coated FastSlide. Binding was revealed by the development of DAB colour reaction.

Figure 6: Binding of anti-dextran antibody to dextran and dextran sulphate.

Different amounts of dextran and dextran sulphate (5 to 500 ng) were applied as 2 mm bands on aluminium oxide membrane (ALOX) and nitrocellulose coated FastSlide. Binding was revealed by the development of DAB colour reaction.

Figure 7: Binding of anti-Le^x antibody to LNFP III as aminopyridine and ADHP derivatives on ALOX and nitrocellulose-coated FastSlide.

Different amounts of oligosaccharide derivatives were applied as 2 mm bands on aluminium oxide membrane (ALOX) and nitrocellulose coated FastSlide. The ADHP (bands 1a-6a) and aminopyridine (PA) derivative

(bands 3b-6b) of the Le^x pentasaccharide LNFP III were detected by an anti-Le^x antibody. The tetrasaccharide LNNT-ADHP was used as negative control (positions 1b and 2b). Binding was revealed by the development of DAB colour reaction

Figure 8: Immobilisation of NGLs on ALOX.

Different amounts of the fluorescent neoglycolipid LNFP III-ADHP were applied on ALOX membrane as 2 mm bands (1, 2, 5, 10, 35 and 70 pmol). After washing with PBS solution at room temperature for 3 hours, the retained NGL on the matrix was measured by its fluorescence and compared with freshly applied unwashed bands. The fluorescent intensities were scanned and shown in (a), and the percentage retained NGL was shown in (b).

Figure 9: Schematic of arrangements in which carbohydrate-containing molecules may be arrayed.

Diagram shows a support according to the first or second aspects of the invention (represented by a rectangle) on which are a number of locations at which carbohydrate-containing molecules may be immobilised (represented by black spots). Each location can be specified according to its position on the support, e.g. A1, A2, B1, B2 etc.

Figure 10: Immobilisation of NGLs on aluminium oxide gel glass slide.

Different amounts of the fluorescent neoglycolipid LNFP III-ADHP were applied on ALOX matrix as 2 mm bands (1, 2, 5, 10, 35 and 70 pmol). After washing with PBS at room temperature for 3 hours, the retained NGL on the matrix was measured by its fluorescence and compared with freshly applied unwashed bands. The fluorescence intensities were scanned and shown in the upper panel, and the percentage of NGL retained shown in the lower panel.

Figure 11: Microarray of NGLs on a nitrocellulose-coated FastSlide

NGLs of LNFP III-DHPE, LNFP II-DHPE and LNT (50 fmol per spot), in 20% n-propanol in water, were arrayed (layout is in panel A) with a touch-type pin microarrayer in the presence of Cy3 dye as a marker (panel B). One slide was stained with an ant-Le^x antibody (panel C) and another with an anti-Le^a (panel D). Binding was detected with a biotinylated anti-mouse followed by Cy5-labelled streptavidin. The LNT was included as a negative control.

Example 1: Aluminium oxide support for carbohydrate arrays.**Introduction**

The invention has been used to generate, to our knowledge, the first arrays with potential to cover the entire range of carbohydrates present in nature (the “glycome”) and in addition chemically synthesized carbohydrates.

The arrays comprise oligosaccharides (including N-glycans, O-glycans, and other free oligosaccharides), glycosaminoglycans (GAGs), glycoproteins, glycolipids, and polysaccharides. Immobilisation/fabrication of carbohydrates are through non-covalent interactions rather than covalent bonding to solid matrices.

As will be described in accompanying examples, the arrays can be used for investigations of protein-carbohydrate interactions, but their use can be extended to interactions between carbohydrates with other molecules. Scientifically, they help to answer the question, at molecular level, if a protein recognises carbohydrate and if so what is the ligand. Due to their comprehensiveness in coverage of carbohydrate sequences, it is possible to

derive the specificities of such interactions. Commercially, they have applications in, for example, drug development.

The aluminium oxide (ALOX) membrane material used here as an example of the support material used in the invention can be prepared on microscope slides. The material has multi-channels and/or multi-layers which lead to increased surface area for better retention of carbohydrates arrayed. Surface modification and coating of the ALOX membrane material are not required. Furthermore, the background fluorescence is low thus signal-to-noise ratio is high.

Materials and Methods

Preparation of carbohydrate-containing molecules.

(1) Oligosaccharides

- *Free reducing oligosaccharides*: any reducing oligosaccharides, such as those isolated from human or animal milk or chemically synthesized.

- *N-linked glycoprotein oligosaccharides*: released by the enzymes peptide-N-(N-acetyl- β -glucosaminyl)asparagine amidase (PNGase F) or endo- β -N-acetylglucosaminidase F (Endo F) or by hydrazinolysis.

- *O-linked glycoprotein oligosaccharides*: released by mild alkaline hydrolysis, by hydrazinolysis and by the sequence-specific enzyme, O-sialoglycoprotein endopeptidase.

- *Natural glycolipids*: released by endo-ceramidase.

- *Proteoglycans or glycosaminoglycans*: released from the proteoglycans or the polysaccharides by lyase digestion or nitrous acid degradation, and in the case of hyaluronic acid, also by hydrolase digestion.

- *Bacterial and plant polysaccharides*: released by partial degradation using various chemical methods, including acid or alkaline hydrolysis, acetolysis, Smith degradation.
- *O-linked mucin-type glycoprotein oligosaccharides*: released by reductive alkaline hydrolysis and containing the GalNAcol core.
- *O-linked mannosyl glycoprotein oligosaccharides*: released by reductive alkaline hydrolysis and containing the Mannol core.
- *Other free oligosaccharide alditols*: reduced for other purposes, *e.g.* for HPLC separation to eliminate the interference caused by the α,β -anomeric forms.

(2) Neoglycolipids (NGLs):

NGLs can be prepared directly from reducing oligosaccharides by reductive-amination with the aminolipid, ADHP or DHPE. NGLs can also be prepared from reduced oligosaccharides by mild periodate oxidation of the open-chain vicinal diol followed by conjugation to the aminolipid, ADHP or DHPE, through reductive-amination.

(3) Other polysaccharides and glycoconjugates (glycolipids, glycoproteins, proteoglycans, glycosaminoglycans and polysaccharides): are isolated by established procedure well known to those skilled in the art.

Arraying of carbohydrate-containing molecules.

Due to the fragile nature of the ALOX membrane, a non-touch type arrayer is preferred for arraying the carbohydrate-containing molecules, *e.g.* a N₂-assisted jet spray as described in the experiments herein.

A water based solvent/solvent mixture is preferred for arraying. Several formulations of water-based solvent mixtures (containing less volatile organic solvents) are being evaluated for arraying NGL with a pin type

microarrays; good results have been obtained with some of the formulations: see Figure 11 for an example of a microarray of NGLs using a solvent based mixture. However, polysaccharides, glycosaminoglycans (GAGs) and glycoproteins can be easily dissolved in water for arraying.

NGL samples were arrayed with N₂-assisted jet spray when in organic-based solvent mixture (chloroform/methanol/water 25:25:8, by vol) as 1-2 mm bands or 150 µm spots. Due to the high sensitivity detection that can be achieved by ALOX membrane, low concentrations of NGLs and glycolipids in water may suffice for arraying and obtaining good binding signals with these oligosaccharide derivatives. When in less volatile solvents NGLs are arrayed with a non-contact arrayer.

Imaging of the results generated from the arrays.

Imaging of the NGLs applied was by fluorescence either directly when fluorescent NGLs were used or after primulin staining when non-fluorescent NGLs were used. Otherwise Cy3 dye is included in the carbohydrate solutions as a tracer at a fixed concentration ratio to the carbohydrate, and thus provides a means of monitoring /imaging the fabricated microarrays.

Binding to the arrayed samples was detected using biotinylated antibodies directed to the proteins being investigated, followed by conventional ELISA-DAB colour reaction or by using Cy5-labelled streptavidin.

Results and Conclusions

The ALOX membrane support material has been compared with nitrocellulose for sensitivities of detection of protein-carbohydrate interactions, using a selection from our carbohydrate collection: ADHPE derivatives of lacto-N-fucopentaose III (LNFP III) and lacto-N-neotetraose

(LNNT); DHPE derivatives of LNFP III, LNNT, 18mer of chondroitin sulphate C (CSC), and 8mer of hyaluronic acid (HA); aminopyridine derivative of LNFP III; and the polysaccharides CSC, Dextran and different preparations of HA. Samples were applied as 1-2 mm bands (larger area for better quantitative analysis and DAB detection). The NGLs used were prepared with an aminolipid 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE) or its fluorescent derivative *N*-aminoacetyl-*N*-(9-anthracenylmethyl)-1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (ADHP).

Comparison of detection sensitivity of NGLs on ALOX and nitrocellulose based support material.

Different amounts (1 fmol to 10 pmol) of fluorescent NGLs were applied as 2 mm bands on ALOX membrane support material and a nitrocellulose coated FastSlide. The arrangement of the NGLs on the support and quantity used is shown under the data. The NGL used was a Lewis^x (Le^x) pentasaccharide LNFP III-ADHP. The presence of the NGL on the support material was detected by an anti-Le^x antibody (Figure 1A). A tetrasaccharide LNNT-ADHP (positions 1b and 2b) was used as the negative control, as this molecule is not recognised by the anti-Le^x antibody.

The binding of the antibody was detected using biotinylated goat anti-mouse immunoglobulins followed by streptavidin-labelled horse radish peroxidase (HRP). Colour development was with fast-diaminobenzidine (DAB). The binding intensity of the antibody to the NGLs was revealed by the DAB colour of each of the bands. The bands were scanned at 550 nm and the colour intensities recorded and plotted for direct comparison (see Figure 1B).

As can be seen from Figure 1, the binding of the antibody to the Lewis^x pentasaccharide on the ALOX membrane support can be detected using 50 to 100 times less carbohydrate than that required to be immobilised on the nitrocellulose based support to give the same detection sensitivity. Therefore, ALOX membrane is a more sensitive support material to use than the nitrocellulose based support material for detecting NGL-molecule binding.

Comparison of detection sensitivity of GAGs on ALOX and nitrocellulose based support material.

Different amounts (5 to 500 ng) of chondroitin sulphate C polysaccharide and its oligosaccharide derived NGL, CSC 18mer-DHPE (0.05 to 5 pmol), were applied as 2 mm bands on ALOX membrane and nitrocellulose coated FastSlide. Chondroitin sulphate C polysaccharide is a glycosaminoglycan (GAG). The presence of the carbohydrate-containing molecules on the support material was detected by an anti-chondroitin sulphate antibody, CS56. Binding of the antibody to the molecules was measured using the DAB colour reaction. The results of this experiment are shown in Figure 2.

Similarly, different amounts of hyaluronic acid polysaccharide (HA) (5 to 500 ng) and its oligosaccharide derived NGL, HA 8mer-DHPE (0.05 to 5 pmol), were applied as 2 mm bands on ALOX membrane and nitrocellulose coated FastSlide. The interactions of TSG-6 (a protein which can bind to the HA) with the carbohydrate-containing molecules on the support material, HA and the HA NGL, are shown in Figure 3.

Also, different hyaluronic acid polysaccharide (HA) preparations (0.5 to 500 ng) were applied as 2 mm bands on ALOX membrane and nitrocellulose coated FastSlide. These were preparations of HA isolated from bovine vitreous humor (HA-BVH) or from Streptococcus (HA-S), and two

preparations of HA from *Streptococcus* with differing molecular weight, 350 kDa (HA-S350) and 220 kDa (HA-S220). The interaction of a biotinylated HA-binding protein (from Calbiochem) to the HA polysaccharides on the support material was detected. by the development of DAB colour reaction. The results of this experiment are shown in Figure 4.

In an additional experiment, different amounts of heparin, heparan sulphate and de-N-acetylated heparin polysaccharide (5 to 500 ng), and chondroitin sulphates A and C together with dextran sulphate (500 ng each) were applied as 2 mm bands on ALOX membrane support and nitrocellulose-coated FastSlide. The carbohydrate-containing molecules were detected by anti-thrombin III (ATIII) binding to the molecules and subsequently ATIII binding was detected using an anti ATIII antibody. Binding of the antibody to the molecules was measured using the DAB colour reaction. The results of this experiment are shown in Figure 5.

As can be seen from Figure 2, the sensitivity of detection of antibody binding was 50-100 times greater with the ALOX membrane support material than the nitrocellulose-based materials. Therefore, ALOX membrane is a more sensitive support material to use than the nitrocellulose based support material for detecting GAG-molecule binding. However, the sensitivity of detection of binding of the antibody to the NGLs derived from CSC oligosaccharides was similar on the support materials.

A similar result can be seen in Figure 3. Based on the signal intensities at the levels tested, ALOX membrane was substantially more sensitive than nitrocellulose based support materials.

Figure 4 shows that at the levels of polysaccharide tested, binding is much stronger with ALOX membrane than the nitrocellulose based support materials.

As can be seen in Figure 5, the ALOX membrane support is substantially more sensitive than the nitrocellulose based support. Antibody binding to the ATIII/heparin complex was undetectable when the heparin was immobilised on the nitrocellulose based Fastslide, while antibody binding to ATIII/heparin complex could be detected when as little as 5 ng of heparin was immobilised on the ALOX membrane support. Similarly, antibody binding to the ATIII/heparin complex could be detected when 5 ng of heparan sulphate was immobilised on the ALOX membrane support, whereas 50 ng had to be immobilised on the nitrocellulose based support before antibody binding to the ATIII/heparin complex could be detected.

The data presented in Figure 5 demonstrates that much less carbohydrate-containing molecules can be immobilised on an ALOX membrane support and antibody binding still be detected. Therefore, ALOX membrane is a suitable support material on which to immobilise carbohydrate-containing molecules.

Therefore, GAGs can be directly immobilised onto ALOX membrane support materials. ALOX membrane is also more sensitive for the detection of carbohydrate-binding than that of the nitrocellulose-based materials.

Binding of anti-dextran antibody to dextran and dextran sulphate.

Different amounts of dextran and dextran sulphate (5 to 500 ng) were applied as 2 mm bands on ALOX membrane and nitrocellulose coated FastSlide. The interaction of an anti-dextran antibody with the carbohydrate-containing molecules on the support material was measured

using the DAB colour reaction. The results of this experiment are shown in Figure 6.

As can be seen from Figure 6, the detection of binding of an antibody to the dextran and dextran sulphate when immobilised on the ALOX membrane support can be 20 to 50 times more sensitive than when the carbohydrate is immobilised on the nitrocellulose based support: on the FastSlide using dextran sulphate there was clear end point at 50 ng whereas on the ALOX membrane end point was not reached at 5 ng. Therefore, ALOX membrane is a more sensitive support material to use than the nitrocellulose based support material for detecting dextran and dextran sulphate-molecule binding.

Binding of anti-Le^x antibody to LNFP III as aminopyridine and ADHP derivatives on ALOX membrane and nitrocellulose-coated FastSlide.

Different amounts of oligosaccharide derivatives were applied as 2 mm bands on ALOX membrane and nitrocellulose coated FastSlide. The arrangement of the NGLs on the support and quantity used is shown under the data. The ADHP (bands 1a-6a) and aminopyridine (PA) derivative (bands 3b-6b) of the Le^x pentasaccharide LNFP III were detected by an anti-Le^x antibody. The tetrasaccharide LNNT-ADHP was used as negative control (positions 1b and 2b). Binding of the antibody to the molecules was measured using the DAB colour reaction. The results of this experiment are shown in Figure 7.

As can be seen in Figure 7, for the neutral oligosaccharide tested as an aminopyridine (PA) derivative, the ALOX membrane support is substantially more sensitive than the nitrocellulose based support: on the FastSlide using PA derivative binding was not detected even at 180 pmol whereas on the ALOX membrane binding could be detected at 45 pmol.

Immobilisation and retention of NGLs on an ALOX membrane

Different amounts of the fluorescent neoglycolipid (NGL) LNFP III-ADHP were immobilised on ALOX membrane as 2 mm bands (1, 2, 5, 10, 35 and 70 pmol). After washing with PBS solution at room temperature for 3 hours, the retained NGL on the matrix was measured by its fluorescence and compared with freshly applied unwashed bands. The fluorescent intensities of immobilised NGLs were scanned and is shown in Figure 8A, and the percentage retained NGL is shown in Figure 8B.

As can be seen in Figure 8, the immobilisation of the fluorescent NGL (LNFP III-ADHP) on the ALOX membrane is good. Also, the retention of the NGL on the matrix after extensive wash is around 60%, even at loadings as high as 100 pmol (Figure 8B).

Example 2: Immobilisation of neoglycolipids on aluminium oxide gel-coated glass slide.

This example demonstrates the utility of a metal oxide gel-coated slide as a support on which to immobilise carbohydrate-containing molecules.

Different amounts of the fluorescent neoglycolipid LNFP III-ADHP (as used in Example 1) were applied on an aluminium oxide gel-coated glass slide as 2 mm bands (1, 2, 5, 10, 35 and 70 pmol). After washing with PBS at room temperature for 3 hours, the retained neoglycolipid on the matrix was measured by its fluorescence and compared with freshly applied unwashed bands. The results of this are presented in Figure 10. The fluorescence intensities were scanned and shown in the upper panel, and the percentage of neoglycolipid retained shown in the lower panel.

The data presented in Figure 10 demonstrates that neoglycolipids can be immobilised on an aluminium oxide gel-coated glass slide and are retained after washing at PBS. Therefore, aluminium oxide gel-coated glass slides are a suitable support material on which to immobilise carbohydrate-containing molecules.

Example 3: A method of detecting a molecule in a test sample

A test sample of body fluid is taken from the patient suspected of suffering from a disorder caused by a microbial infection.

A support according to the first or second aspects of the invention is prepared having immobilised a carbohydrate-containing molecule or molecules to which a molecule indicative of the presence of a specific microbial infection will bind. For example, the molecule could be an antibody which recognises an epitope contained within the immobilised carbohydrate-containing molecule.

The test sample is applied to the support and the binding of a molecule in the test sample to a carbohydrate-containing molecule immobilised on the support is then measured using, for example, a fluorescence detection system. From the data generated it is possible to determine whether the patient is suffering from a microbial infection and, hence, benefit from treatment.

Example 4: A method to identify whether a molecule binds to a carbohydrate-containing molecule.

An array of carbohydrate-containing molecules representing the carbohydrate composition of a specific cell type or tissue (in this example a

human brain) is prepared on a support according to the first or second aspects of the invention. Hence the array is a complex mixture of carbohydrate-containing molecules representing the glycome of a human brain. The carbohydrate-containing molecules in this example are neoglycolipids.

The array is contacted with a quantity of a molecule, for example a possible therapeutic molecule, and any binding of a molecule in the test sample to a the array of neoglycolipids is measured using, for example, a fluorescence detection system. From the data generated it is possible to determine whether the possible therapeutic molecule interacts with a component of the glycome of the human brain. Such a method may be of particular use in screening possible therapeutic molecules to identify molecules which may be suitable for further analysis.

Example 5: A method of measuring the kinetics of interaction between a molecule and a carbohydrate-containing molecule.

An array of carbohydrate-containing molecules is prepared on a support according to the first aspect of the invention. The carbohydrate-containing molecules arrayed are identical, however the quantity of carbohydrate-containing molecules varies at different locations throughout the array.

The array is contacted with a quantity of a molecule known to interact with the arrayed carbohydrate-containing molecule. The kinetics of interaction of the molecule to the carbohydrate-containing molecule can be measured by real time changes in colorimetric or fluorescent signals.

Such a method may be of particular use in, for example, identifying a therapeutic molecule which can bind with a high affinity to carbohydrate-containing molecules.

Example 6: A method to identify a carbohydrate-binding molecule in a heterogeneous population of molecules.

An array of carbohydrate-containing molecules representing the carbohydrate composition of a specific cell type or tissue (in this example a human brain) is prepared on a support material according to the first or second aspects of the invention. Hence the array is a complex mixture of carbohydrate-containing molecules representing the glycome of a human brain. The carbohydrate-containing molecules in this example are neoglycolipids.

The array is contacted with a quantity of a heterogeneous population of molecules, for example a possible therapeutic molecules, and binding of any of the molecules to the array of neoglycolipids is measured using, for example, a fluorescence detection system. From the data generated it is possible to identify possible therapeutic molecules from a heterogeneous population of molecules which may be suitable for further analysis.

Example 7: A method to identify a carbohydrate bound by a molecule (optionally from a heterogeneous population of carbohydrates).

An array of carbohydrate-containing molecules representing the carbohydrate composition of a specific cell type or tissue (in this example a human brain) is prepared on a support according to the first or second aspects of the invention. Hence the array is a complex mixture of carbohydrate-containing molecules representing the glycome of a human

brain. The carbohydrate-containing molecules in this example are neoglycolipids.

The array is contacted with a quantity of a molecule, for example a possible therapeutic molecule, and binding of the molecule to the array of neoglycolipids is measured using, for example, a fluorescence detection system.

Should a complex mixture of neoglycolipids be identified as binding to the molecule, then the specific neoglycolipid in this population which binds to the molecule can be identified.

One method of identifying the specific neoglycolipid from a complex population of neoglycolipids is to use a deconvolution strategy. For example, a 'daughter' array of the complex mixture of neoglycolipids separated into individual or a restricted number of molecules can be generated. The molecule is then used to screen the daughter array and any neoglycolipids to which the molecule binds can be identified using mass spectrometry preceded as necessary by thin-layer or multi-dimensional chromatographies and chromatogram binding.

Example 8: A method of separating microbes or specific cells from biological samples.

A support according to the first or second aspects of the invention is prepared, on which is immobilised carbohydrate-containing molecules which is used to identify microbes, for example, from the urine of a patient having, or suspected of having, a urinary infection caused by a bacterium.

Further examples of this method of 'panning' for specific microbes or cells, for example virally infected cells, in a heterogeneous population will be obvious to a person skilled in the art. Also possible are methods for identifying other microbes, such as viruses, using the same principle as that outlined above.

Example 9: A method to identify whether a molecule interferes with the binding of a molecule or cell to a carbohydrate-containing molecule.

A support according to the first or second aspects of the invention is prepared having immobilised a homogenous population of carbohydrate-containing molecules, in this case neoglycolipids. The support is then exposed to a quantity of a molecules which binds with the immobilised neoglycolipids.

Once prepared, the support having the neoglycolipid/molecule complex is then contacted with a quantity of a test molecule, in this example a small drug. Any interference in the binding of the molecule to the neoglycolipid by the small drug can be measured by, for example, detecting changes in the quantity of molecule binding to the neoglycolipid.

The method described above is a competition/inhibition assay which, as would be appreciated by a person skilled in the art, could be the basis for a screen to identify possible therapeutic molecules which affect the binding of, for example, a microbe to a carbohydrate-containing molecule present on a cell surface. The screen can be modified such that pools of test molecules can be screened to identify a test molecule which interferes with the binding of a molecule to a carbohydrate-containing molecule from a heterogeneous population of test molecules, as will be appreciated by a person skilled in the art

Claims

1. A support on which is immobilised a carbohydrate-containing molecule or molecules,
wherein said support has multiple channels substantially perpendicular to the surface of the support onto which the carbohydrate-containing molecule or molecules is applied, and
wherein the channels are open to the surface and have a diameter large enough to accommodate the carbohydrate-containing molecule.
2. A support defined in claim 1 which has channels of between 0.01 and 5 μm in diameter.
3. A support as defined in claim 2 which has channels of 0.2 μm in diameter.
4. A support as defined in any of the preceding claims which has channels at a density of 1 to 100 channels / μm^2 .
5. A support as defined in claim 4 which has channels at a density of between 32 to 38 channels / μm^2 .
6. A support as defined in any of the preceding claims which comprises a metal oxide.
7. A support as defined in claim 6 which comprises an electrochemically manufactured metal oxide membrane.
8. A support as defined in claim 6 or 7 wherein the metal oxide is aluminium oxide.

9. A support on which is immobilised a carbohydrate-containing molecule or molecules, wherein said support comprises a metal oxide.
10. A support as defined in claim 9 which comprises a metal oxide gel.
11. A support as defined in claim 8 or 9 wherein the metal oxide is aluminium oxide.
12. A support as defined in any of the preceding claims wherein the immobilised carbohydrate-containing molecule or molecules are arrayed on the support.
13. A support as defined in any of the preceding claims wherein the carbohydrate-containing molecule or molecules is a polysaccharide.
14. A support as defined in any of claims 1 to 12 wherein the carbohydrate-containing molecule or molecules is a glycolipid.
15. A support as defined in any of claims 1 to 12 wherein the carbohydrate-containing molecule or molecules is a glycosaminoglycan or an oligosaccharide fragment of a glycosaminoglycan.
16. A support as defined in any of claims 1 to 12 wherein the carbohydrate-containing molecule or molecules is a glycoprotein.

17. A support as defined in any of claims 1 to 12 wherein the carbohydrate-containing molecule or molecules is a reducing end-tagged molecule.
18. A support as defined in claim 17 wherein the reducing end-tagged molecule or molecules is a neoglycolipid.
19. A support as defined in claim 17 wherein the reducing end-tagged molecule or molecules is a glycolipid.
20. A support as defined in claim 18 wherein the neoglycolipid comprises a tag of between 24 to 50 carbon atom length.
21. A support as defined in claim 17 wherein the reducing end-tagged molecule comprises a tag of between 5 to 25 carbon atoms with an aliphatic or aromatic hydrocarbon backbone.
22. A support as defined in claims 18 to 20 wherein the neoglycolipid or glycolipid has a chromophore.
23. A support as defined in claims 17 to 22 wherein the carbohydrate is an oligosaccharide.
24. A support as defined in claims 17 to 22 wherein the carbohydrate is a monosaccharide.
25. A support as defined in claim 23 wherein the oligosaccharide is an *N*-glycan.

26. A support as defined in claim 23 wherein the oligosaccharide is an *O*-glycan.
27. A support as defined in claim 26 wherein the *O*-glycan terminates in *N*-acetylgalactosamine or *N*-acetylgalactosaminitol.
28. A support as defined in claim 26 wherein the *O*-glycan terminates in mannose or mannitol.
29. A support as defined in claim 23 wherein the oligosaccharide is a GPI-linked glycan.
30. A support as defined in claim 23 wherein the oligosaccharide is a fragment of a glycosaminoglycan.
31. A support as defined in claims 23 to 30 wherein the oligosaccharide or monosaccharide is derived from one or more carbohydrate sources selected from glycoproteins, glycolipids, proteoglycans/glycosaminoglycans and polysaccharides or is synthesised chemically.
32. A support as defined in claim 31 wherein the oligosaccharide or monosaccharide is a reducing sugar.
33. A support as defined in claim 31 wherein the oligosaccharide or monosaccharide is a reduced sugar.
34. A support as defined in claim 33 wherein the reduced oligosaccharide or monosaccharide is tagged at the reducing terminal after a mild oxidation procedure.

35. A support as defined in any of the preceding claims wherein there are one or more samples of carbohydrate-containing molecules which comprise a homogeneous sample of carbohydrate.
36. A support as defined in any of claims 1 to 34 wherein there are one or more samples of carbohydrate-containing molecules which comprise a heterogeneous sample of carbohydrate.
37. A support as defined in any of the preceding claims wherein the carbohydrate-containing molecule or molecules comprises carbohydrate derived from a microbe.
38. A support as defined in any of claims 1 to 36 wherein the carbohydrate-containing molecule or molecules comprises carbohydrate derived from a specific cell type.
39. A support as defined in any of claims 1 to 36 wherein the carbohydrate-containing molecule or molecules comprises carbohydrate derived from a specific tissue or organ.
40. A support as defined in claims 38 or 39 where the cell type or tissue or organ is derived from an animal.
41. A support as defined in claim 40 where the animal is a human.
42. A support as defined in claims 38 or 39 where the cell type or tissue or organ is derived from a plant.

43. A method of preparing a support according to any of the preceding claims wherein the carbohydrate-containing molecule or molecules is immobilised on the support while solubilised in a solvent comprising an aqueous/aliphatic alcohol mixture.
44. The method of claim 43 wherein said support is the support according to claims 14, 17 to 19 or any of claims 20 to 42 when dependent on claims 14, 17 to 19.
45. The method of claim 43 or 44 wherein said solvent includes between 8 to 15% (v/v) of an aliphatic alcohol selected from a list comprising: propanol (propan-1-ol), iso-propanol (propan-2-ol), n-butanol (butan-1-ol), iso-butanol (butan-2-ol), and t-butanol (2-methylpropan-2-ol).
46. A method for detecting a molecule in a test sample comprising:
 - i) contacting a support according to any of the preceding claims with the test sample; and,
 - ii) detecting the binding of any molecules in the test sample to the carbohydrate-containing molecule or molecules.
47. A method of determining whether a molecule interacts with a carbohydrate comprising:
 - i) contacting a support according to any of the preceding claims with the molecule; and

- ii) measuring whether the molecule binds to a carbohydrate-containing molecule or molecules on the support.
48. The method of claim 47 wherein the support is as defined in claim 12.
49. A method of determining the kinetics of interaction between a molecule and a carbohydrate comprising:
- i) contacting a support according to any of the preceding claims with a molecule; and
 - ii) measuring the kinetics of interaction between the molecule and a carbohydrate-containing molecule or molecules on the support.
50. The method of claim 49 wherein the support is as defined in claim 12.
51. The method of claim 49 wherein the support is as defined in any one of claims 1 to 8 or claims 12 to 42 when dependent on claims 1 to 8.
52. A method of identifying a carbohydrate-binding molecule or molecules in a heterogeneous sample of molecules comprising:
- i) contacting a support as defined in any of the preceding claims with a heterogeneous sample of molecules; and
 - ii) identifying a molecule or molecules which interact with a carbohydrate-containing molecule or molecules on the support.

53. The method of claim 52 wherein the support is as defined in claim 12.
54. A method of identifying a carbohydrate bound by a molecule (optionally from a heterogeneous population of molecules) comprising:
- i) contacting a support according to any of the preceding claims with the molecule; and
 - ii) identifying the carbohydrate-containing molecule or molecules on the support to which the molecule binds.
55. The method of claim 54 wherein the support is as defined in claim 12.
56. The method of claim 54 or claim 55 wherein part (ii) further comprises a deconvolution process.
57. A method of separating specific cells from a heterogeneous population of cells comprising:
- i) providing a support as defined in any of the preceding claims, wherein the carbohydrate-containing molecule or molecules is able to interact with specific cells;
 - ii) contacting the support with a heterogeneous population of cells; and

iii) separating those cells that bind to the carbohydrate-containing molecule or molecules from those cells that do not bind to the carbohydrate-containing molecule or molecules on the support.

58. A method of determining whether a test molecule interferes with the binding of a molecule or cell to a carbohydrate-containing molecule comprising:

i) providing a support as defined in any of the preceding claims, wherein the carbohydrate-containing molecule or molecules is bound by a molecule or cell;

ii) contacting the support with a test molecule; and

iii) identifying whether the test molecule interferes with the binding of a molecule or cell to the carbohydrate-containing molecule or molecules on the support.

59. The method of claim 58 wherein the test molecule is part of a heterogeneous population of molecules.

60. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a polypeptide.

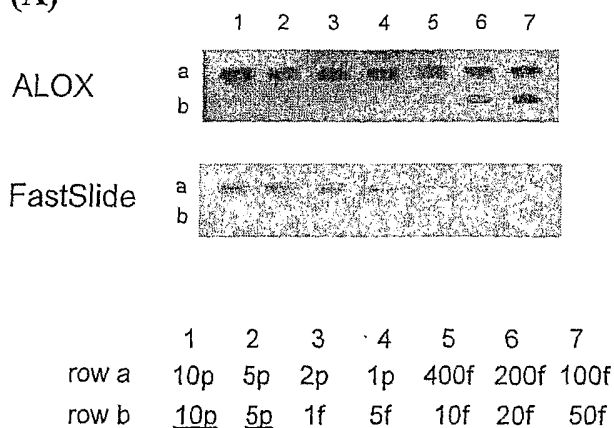
61. A method as defined in claim 60 wherein the polypeptide is an antibody.

62. A method as defined in claim 60 wherein the polypeptide is an enzyme.

63. A method as defined in claim 60 wherein the polypeptide is a receptor.
64. A method as defined in claim 60 wherein the polypeptide is a lectin.
65. A method as defined in claim 60 wherein the polypeptide is a glycoprotein.
66. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a peptidomimetic.
67. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a nucleic acid
68. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a carbohydrate.
69. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a lipid.
70. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a glycolipid.
71. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a hormone.
72. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a microbial antigen.

73. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a therapeutic molecule.
74. A method as defined in claim 73 wherein the therapeutic molecule is smaller than 500 daltons.
75. A method as defined in claims 73 or 74 wherein the therapeutic molecule is a prophylactic agent
76. A method as defined in claims 73 or 74 wherein the therapeutic molecule is a vaccine.
77. A method as defined in claims 73 or 74 wherein the therapeutic molecule is an immunomodulator.
78. A method as defined in claims 46 to 56, 58 and 59 wherein the molecule or test molecule is a glycomimic.
79. A molecule as identified by the method of any of claims 47, 48, 52, 53, 58 or 59.
80. A carbohydrate as identified by the method of claims 54 to 56.
81. Use of a solvent comprising an aliphatic alcohol for solubilising a neoglycolipid and/or a glycolipid in the preparation of a support according to any of the preceding claims.
82. The use according to claim 81 wherein said support is the support according to claims 14, 17 to 19 or any of claims 20 to 42 when dependent on claims 14, 17 to 19.

83. The use according to claim 81 or 82 wherein the solvent includes between 8 to 15% (v/v) of an aliphatic alcohol selected from a list comprising: propanol (propan-1-ol), iso-propanol (propan-2-ol), n-butanol (butan-1-ol), iso-butanol (butan-2-ol), and t-butanol (2-methylpropan-2-ol).

Figure 1**(A)**

LNFP III-ADHP was used as an example and
 LNNT-ADHP (underlined) as negative control;
 p: pmol, f: fmol.

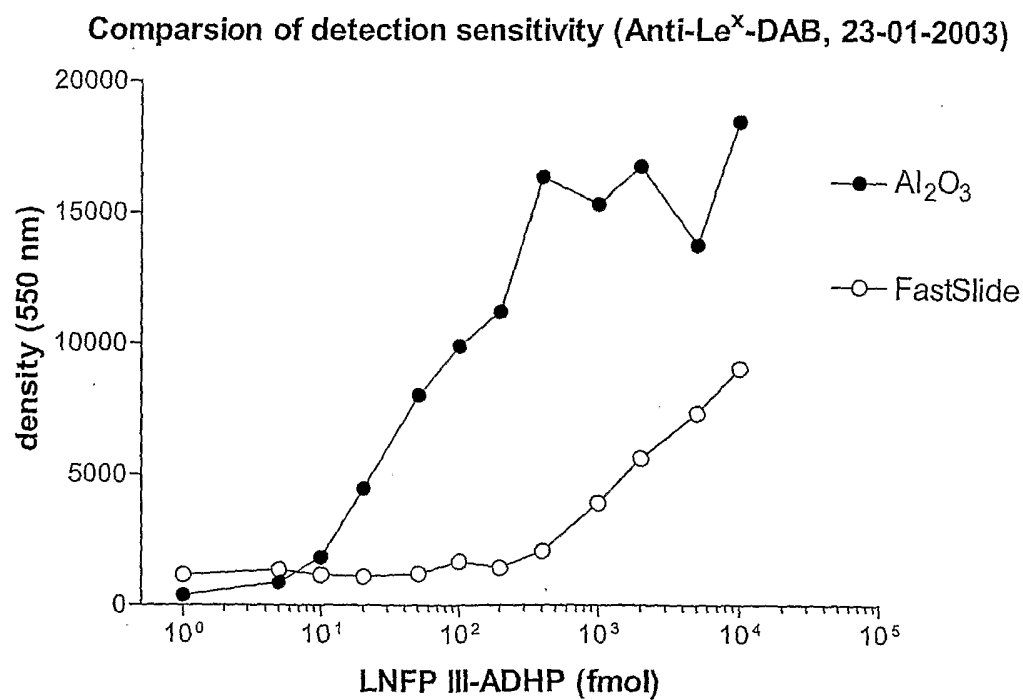
(B)

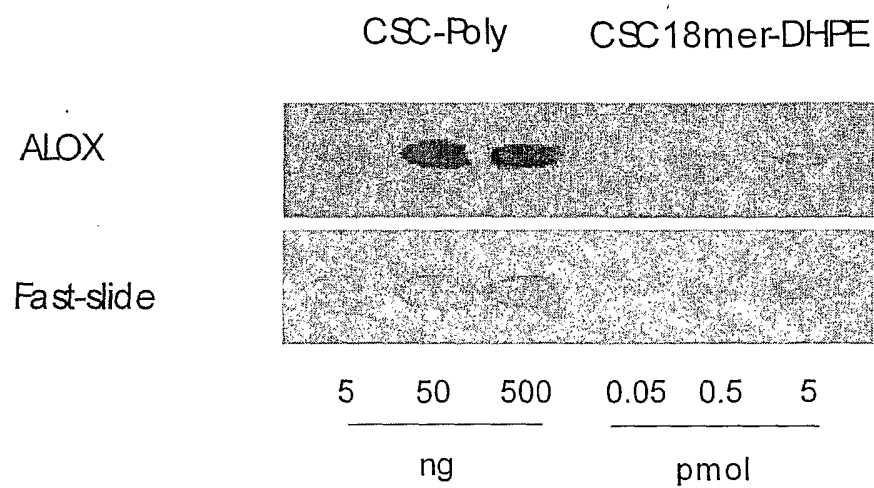
Figure 2

Figure 3

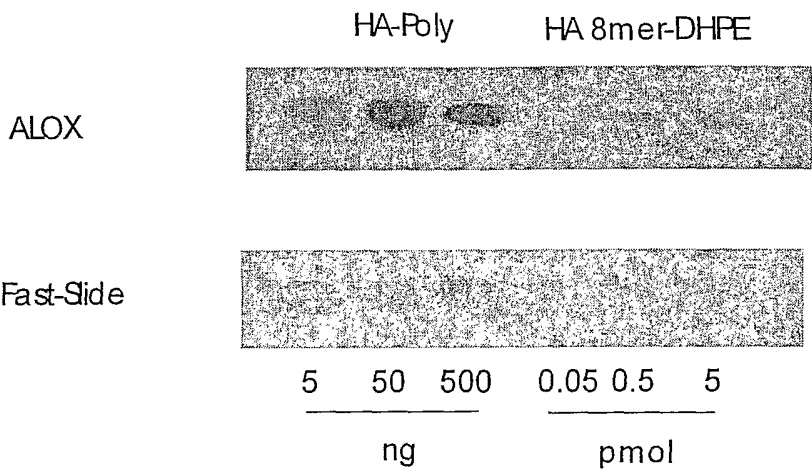


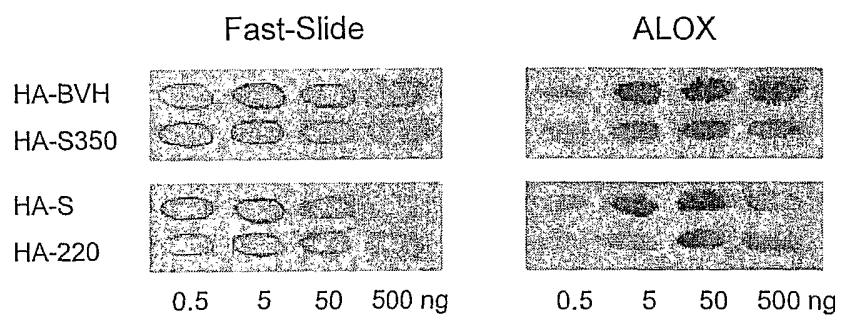
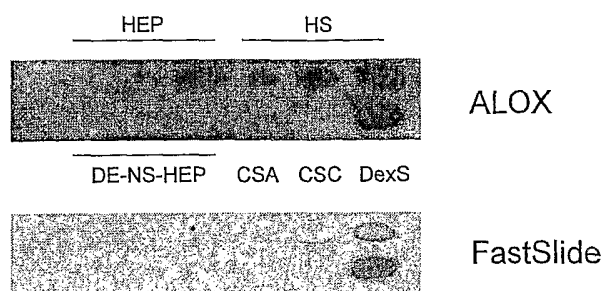
Figure 4

Figure 5



Anti thrombin III: 30ug/ml

CSA, CSC, dextran sulphate (DexS): 500 ng

HEP, HS and De-N-acetylated HEP: 5, 50, and 500 ng from left to right.

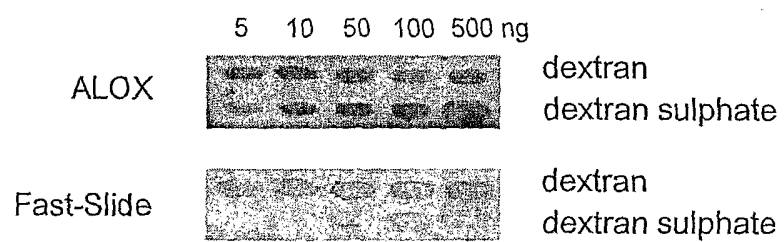
Figure 6

Figure 7

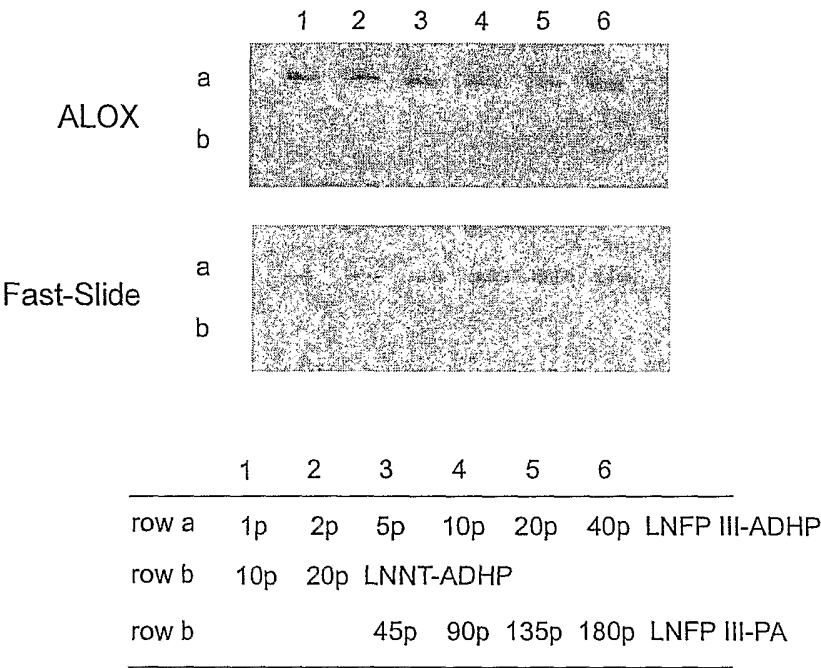


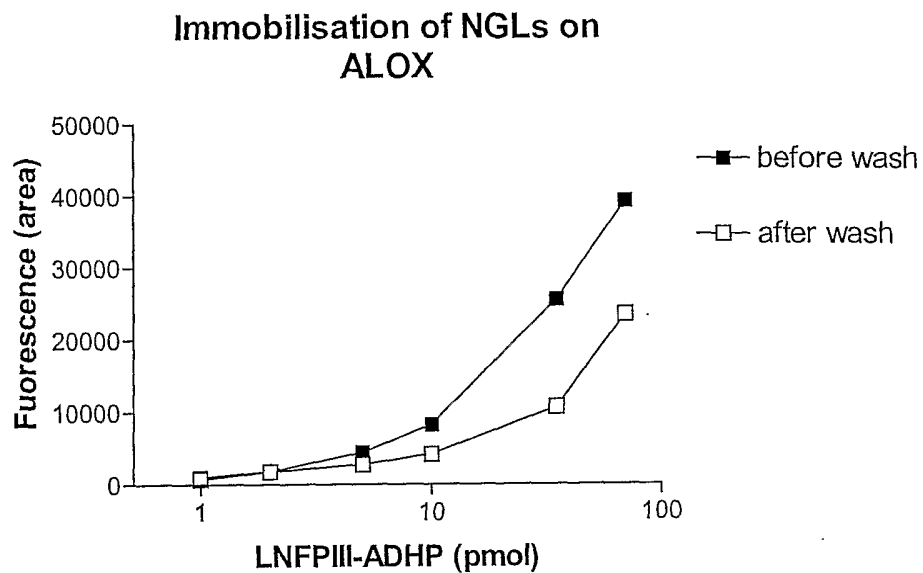
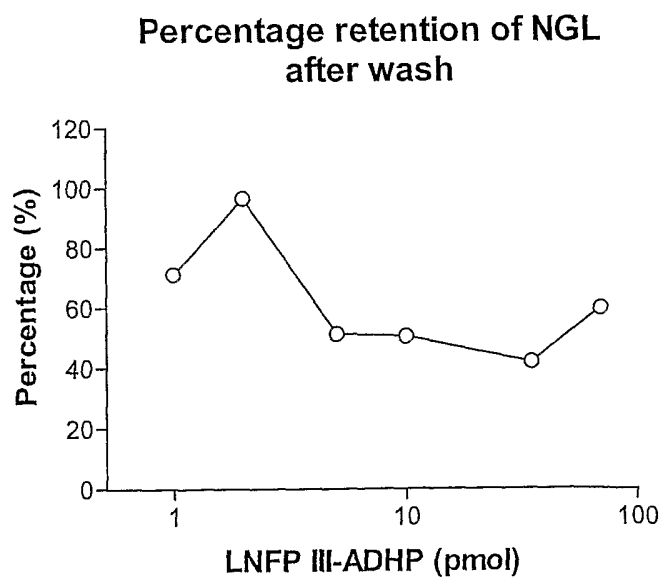
Figure 8**(A)****(B)**

Figure 9

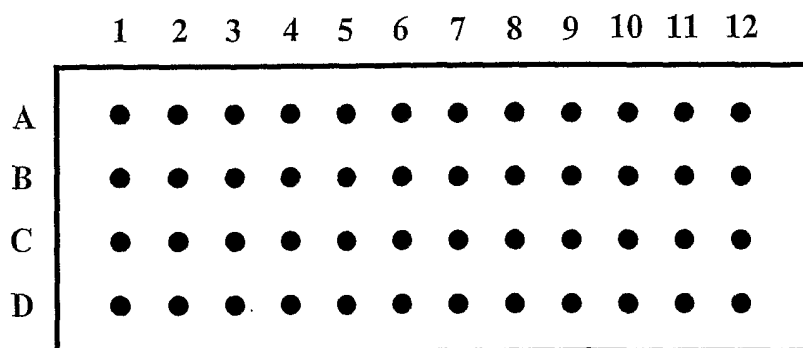


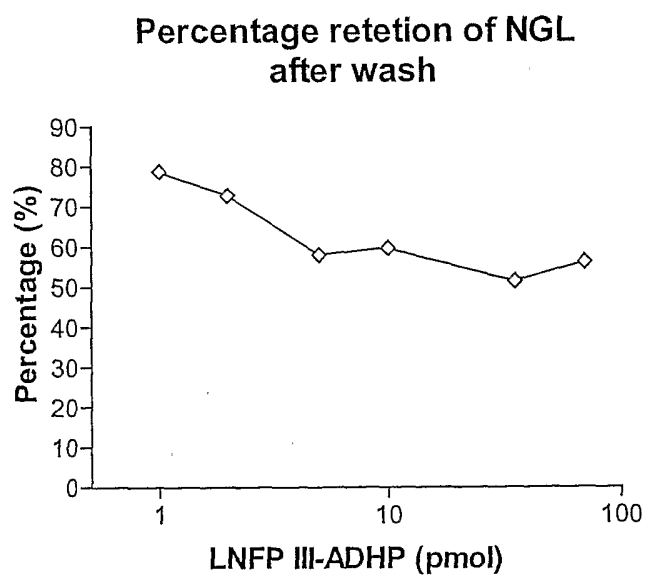
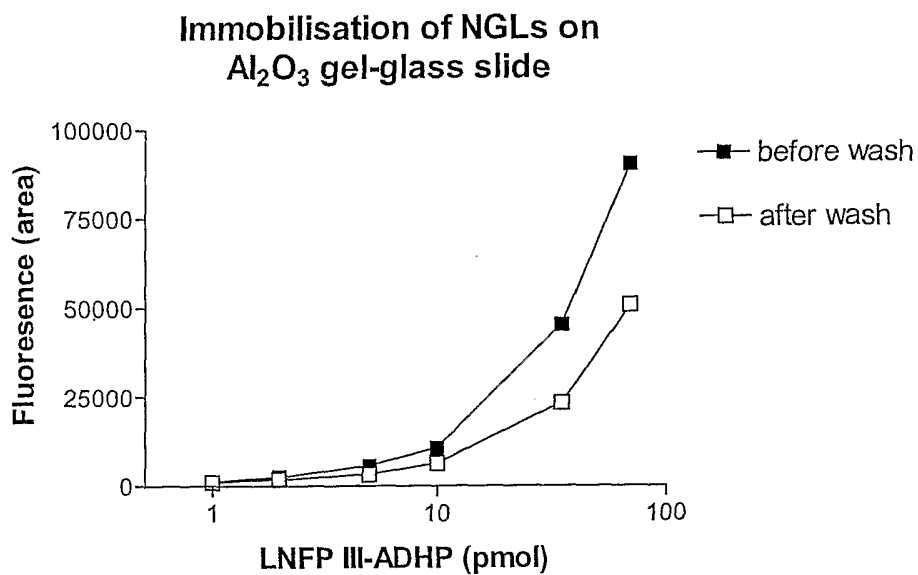
Figure 10

Figure 11

Microarray of lacto-N-tetraose (LNT),
lacto-N-fucopentaose-II and -III (LNFP-II & LNFP-III)

