

PRINTING OF FSL CONSTRUCTS**FIELD OF INVENTION**

The invention relates to a method of printing constructs of the generic structure F-S-L (where F is a functional moiety, S is a
5 spacer covalently linking F to L, and L is a lipid).

In particular, the invention relates to the use of the method in the fabrication of diagnostic test cards and sticks, microarrays and multiwell plates.

BACKGROUND ART

10 Glycomics has emerged with proteomics as an area for development and exploration in the postgenomics era (Blixt et al (2004)). Despite the increasing awareness of the biological significance of carbohydrates, the study of carbohydrate-protein interactions still encounters much difficulty. There is a need for the
15 development of highly sensitive and high-throughput methods for identification and binding study of carbohydrates recognized by various receptors (Chung-Yi et al (2009)).

The immobilization of glycans on the derivatised surface of substrates is a commonly employed method of fabricating glycan
20 microarrays. Blixt et al (2004) discloses immobilisation of amine functionalised synthetic glycan ligands on N-hydroxysuccinimide (NHS) activated glass slides using a custom made robotic printing arrayer. Bovin and Huflejt (2008) have reviewed the use of binding chemistries exploiting amide bond formation. Short
25 spacers are used to reduce non-specific contacts to a minimum. Attachment to a flexible layer of polyethylene glycol on a glass surface is presented as assuring availability of glycan moieties for interaction with binding molecules.

The localization of glycans to the surface of substrates in the
30 form of neoglycolipids has also been employed as a method of fabricating glycan microarrays. Chai et al (2003) describe a multiwell-binding assay in which neoglycolipids are diluted either in methanol, or in methanol containing the carrier lipids egg

- lecithin and cholesterol. The dispersions of neoglycolipids are then used to coat the wells of the multiwell plates. Chai *et al* (2004) describe the bandwise application of the dispersions of neoglycolipids by a spray-on technique employing a sample applicator comprising a single syringe as applicator (LINOMAT IV, Camag, Switzerland)
- Fukui *et al* (2005) and Huang *et al* (2006a, 2006b) have each described a non-covalent glycoarray assembly method utilising lipid-linked saccharides and oligosaccharides. Both methods employ reductive amination to produce a lipid-linked saccharide of oligosaccharide (neoglycolipid). In the method of Fukui *et al* (2005) oligosaccharides were conjugated to 1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine (DHPE) directly or after mild periodate oxidation. The neoglycolipids were then applied by jet spray as bands or spots onto nitrocellulose membranes. In the method of Huang *et al* (2006a, 2006b) the reaction to produce lipid-linked saccharides uses an excess of saccharides in order to exhaust the tetradecylamine employed in the reaction. The lipid-linked saccharides were then applied to multi-well high binding polystyrene plates.
- Liu *et al* (2006) describe the preparation of neoglycolipids from N-aminoxyacetal DHPE (AOPE) by a chemoselective oxime-ligation reaction with reducing sugars. The binding of the neoglycolipids by antibodies and lectins was assayed by an enzyme-linked immunosorbent assay (ELISA) in plastic microwells as described by Chai *et al* (2003). In these studies the neoglycolipids were incorporated into liposomes for arraying and spotted onto nitrocellulose membranes or robotically arrayed onto nitrocellulose-coated glass slides.
- Palma *et al* (2006) and Campanero-Rhodes *et al* (2007) describe the preparation of arrays of natural and synthetic glycolipids and neoglycolipids by printing on nitrocellulose-coated glass slides using a non-contact piezoelectric arrayer (PIEZORRAY, Perkin-Elmer, United Kingdom). Liu *et al* (2007) also describes the use of this non-contact piezoelectric arrayer. The arrayer employs an

assembly containing four PIEZOTIP™ dispensers to dispense sub-nanolitre to nanolitre volumes with 20 to 25 µm accuracy and precision.

5 It is an object of the invention to provide an improved method for the localisation of functional moieties, including glycans, to the surface of substrates.

It is an object of the invention to provide a method of fabricating diagnostic test cards and sticks, microarrays and multiwell plates.

10 It is an object of the invention to provide templates for use in the fabrication of diagnostic test cards and sticks, microarrays and multiwell plates microarray formats by the method that improve accuracy and reliability of assay results.

15 These objects are each to be read disjunctively with the object to at least provide the public with a useful choice.

STATEMENT OF INVENTION

In a **first** aspect the invention provides a method of localising a functional moiety (F) to at least one discrete area on a surface of a substrate including the step of propelling droplets of a dispersion of a synthetic construct of the structure F-S-L from a plurality of orifices located in a monolithic print head onto the surface of the substrate where:

- S is a spacer (S) selected to provide a construct that is dispersible in water in the absence of organic solvents or detergents at a temperature of 25 °C; and
- L is a diacyl- or dialkyl lipid.

Preferably, F is biotin, a glycan or a peptide.

In a first preferment of the first aspect of the invention, the at least one discrete area is in the shape of a symbol. More preferably, the at least one discrete area is in the shape of a symbol readable by optical character recognition (OCR) apparatus.

Most preferably, the at least one discrete area is in the shape of a symbol comprising one or more alphanumeric characters. In a second preferment of the first aspect of the invention, the at least one discrete area is a pattern comprising a combination of
5 indicia in which the dispersion of a synthetic construct is present at different densities (amount per unit area). The first and second preferments of this aspect of the invention are not mutually exclusive.

Preferably, the substrate is selected from the group consisting
10 of: derivatised silica gel (e.g. C₈ or C₁₈), nitrocellulose, coated paper, silica gel or uncoated paper. More preferably, the substrate is selected from the group consisting of: coated paper or uncoated paper.

The method is a non-impact method of printing. The propelling
15 droplets from a plurality of orifices is from a plurality of orifices located in a monolithic print head. Preferably, the propelling droplets from a plurality of orifices is from a plurality of orifices located in a monolithic print head of an inkjet printer. Most preferably, the propelling droplets from a
20 plurality of orifices is from a plurality of orifices located in a monolithic print head of a piezoelectric inkjet printer.

Preferably, the volume of each of the droplets is 1 to 100 picolitres (pL). More preferably, the volume of each of the droplets is 1 to 50 pL. Most preferably, the volume of each of
25 the droplets is 1 to 5 pL.

Preferably, the concentration of the synthetic construct in the dispersion is 1 μ molar (μ M) to 10 mmolar (mM). More preferably, the concentration of the synthetic construct in the dispersion is 10 μ M to 10 mM. Most preferably, the concentration of the
30 synthetic construct in the dispersion is 0.1 to 10 mM.

Preferably, the synthetic construct of the structure F-S-L is dispersible in water in the absence of organic solvents or detergents at a temperature of 25 °C at a concentration of at least 6 millimolar (mM). More preferably, the synthetic construct

of the structure F-S-L is dispersible in water in the absence of organic solvents or detergents at a temperature of 25 °C at a concentration of at least 12 millimolar (mM).

Preferably, L is a glycerophospholipid. More preferably, L is a phosphatidylethanolamine. Most preferably, L is selected from the group consisting of: 1,2-O-diioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-O-distearyl-sn-glycero-3-phosphatidylethanolamine (DSPE).

In a first preferment of the first aspect of the invention F is a glycan. Preferably, F is a glycan that is an oligosaccharide. More preferably, F is a glycan that is an oligosaccharide selected from the group consisting of: GalNAc α 3(Fuc α 2)Gal β -;
Gal α 3(Fuc α 2)Gal β -; GalNA α 3(Fuc α 2)Gal β -; Fuc α 2Gal β -;
Gal β 4GlcNAc β 3(Gal β 4GlcNAc β 6)Gal β -; Gal β 4GlcNAc β 3-; Gal β 4Glc β -;
Gal β 3GlcNAc β -; Gal β 3(Fuc α 4)GlcNAc β -; Fuc α 2Gal β 3(Fuc α 4)GlcNAc β -;
GalNAc α 3(Fuc α 2)Gal β 3(Fuc α 4)GlcNAc β -;
Gal α 3(Fuc α 2)Gal β 3(Fuc α 4)GlcNAc β -; Gal β 4(Fuc α 3)GlcNAc β -;
Fuc α 2Gal β 4(Fuc α 3)GlcNAc β -; NeuAc α 2-3Gal β 3(Fuc α 4)GlcNAc β -; NeuAc α 2-3Gal β 4(Fuc α 3)GlcNAc β -; GalNAc β 4(NeuAc α 2-3)Gal β 4-; Gal β 3GalNAc α -;
NeuAc α 2-3Gal β 4-; NeuAc α 2-6Gal β 4-; Gal α 4Gal β 4-; GalNAc β 3Gal α 4Gal β 4-;
Gal α 4Gal β 4GlcNAc β 3-; Gal β 3GalNAc β 3Gal α 4-; NeuAc α 2-3Gal β 3GalNAc β 3Gal α 4-; Gal α 3Gal β -; GalNAc α 3GalNAc β 3Gal α 4-;
GalNAc β 3GalNAc β 3Gal α 4-; Gal β 1-4GlcNAc; Gal β 1-3GlcNAc; SA α 2-6Gal β 1-4Glc; SA α 2-3Gal β 1-4Glc; SA α 2-6Gal β 1-4GlcNAc; SA α 2-3Gal β 1-4GlcNAc;
SA α 2-3Gal β 1-3GlcNAc; Gal β 1-4(Fuc α 1-3)GlcNAc; Gal β 1-3(Fuc α 1-3)GlcNAc; SA α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc; SA α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc; Gal β 1-4GlcNAc β 1-4GlcNAc; Gal β 1-3GlcNAc β 1-4GlcNAc; SA α 2-6Gal β 1-4GlcNAc β 1-4GlcNAc; SA α 2-3Gal β 1-4GlcNAc β 1-4GlcNAc; SA α 2-3Gal β 1-3GlcNAc β 1-4GlcNAc; Gal β 1-4(Fuc α 1-3)GlcNAc β 1-4GlcNAc; Gal β 1-3(Fuc α 1-4)GlcNAc β 1-4GlcNAc; SA α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-4GlcNAc; SA α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-4GlcNAc; SA α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-4Gal; SA α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-4Gal; SA α 2-3Gal β 1-

4GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc; SA α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-
4(Fuca1-3)GlcNAc; SA α 2-3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc;
SA α 2-6Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-3Gal β 1-4GlcNAc β 1-
3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-
5 4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-3Gal β 1-4(Fuca1-3)GlcNAc β 1-
3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-6Gal β 1-4(Fuca1-
3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-3Gal β 1-
4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc; SA α 2-6Gal β 1-4(Fuca1-
3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc; SA α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-
10 4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc; SA α 2-6Gal β 1-4GlcNAc β 1-
3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc; SA α 2-3Gal β 1-
4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc;
SA α 2-6Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-
4(Fuca1-3)Glc; SA α 2-3Gal β 1-3(Fuca1-4)GlcNAc; SA α 2-6Gal β 1-3(Fuca1-
15 4(GlcNAc; SA α 2-3Gal β 1-3GlcNAc β 1-4Gal β 1-4(Fuca1-3)GlcNAc; SA α 2-
6Gal β 1-3GlcNAc β 1-4Gal β 1-4(Fuca1-3)GlcNAc; SA α 2-3Gal β 1-3(Fuca1-
4)GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc; SA α 2-6Gal β 1-3(Fuca1-4)GlcNAc β 1-
3Gal β 1-4(Fuca1-3)GlcNAc; SA α 2-3Gal β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-
4Glc; SA α 2-6Gal β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-3Gal β 1-
20 3GlcNAc β 1-4Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-6Gal β 1-
3GlcNAc β 1-4Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc; SA β 2-3Gal β 1-
3(Fuca1-4)GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-
6Gal β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc;
SA α 2-3Gal β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc; SA α 2-6Gal β 1-
25 3(Fuca1-4)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc; SA α 2-3Gal β 1-3GlcNAc β 1-
4Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc; SA α 2-6Gal β 1-
3GlcNAc β 1-4Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc; SA α 2-
3Gal β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-
3)Glc; SA α 2-6Gal β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-4(Fuca1-3)GlcNAc β 1-
30 3Gal β 1-4(Fuca1-3)Glc; SA α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-3(Fuca1-
4)GlcNAc; SA α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-3(Fuca1-4)GlcNAc; SA α 2-
3Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-3(Fuca1-4)GlcNAc; SA α 2-6Gal β 1-

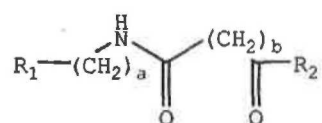
4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-3(Fuc α 1-4)GlcNAc; SA α 2-3Gal β 1-4GlcNAc β 1-
3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-
3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-
3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-6Gal β 1-4(Fuc α 1-
5 3)GlcNAc β 1-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc; SA α 2-3Gal β 1-
4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-3(Fuc α 1-4)Glc; SA α 2-6Gal β 1-4(Fuc α 1-
3)GlcNAc β 1-3Gal β 1-3(Fuc α 1-4)Glc; SA α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-
3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc; SA α 2-6Gal β 1-4GlcNAc β 1-
3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc; SA α 2-3Gal β 1-
10 4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc;
and SA α 2-6Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-
3Gal β 1-4(Fuc α 1-3)Glc, where SA is sialic acid.

In a second preferment of the first aspect of the invention F is a
peptide. More preferably, F is a peptide that is an oligopeptide.
15 Most preferably, F is a peptide selected from the group listed in
the *Table of Peptides*.

In a third preferment of the first aspect of the invention F is a
conjugator. More preferably, F is a conjugator that is biotin.
When F is a conjugator that is biotin, the biotin may or may not be
20 conjugated to an avidinylated functional moiety.

Preferably, the method includes the step of coating the surface of
the substrate with a polymer after the propelling of the droplets
of the dispersion of the synthetic construct of the structure F-S-
L onto the surface of the substrate. More preferably, the method
25 includes the step of coating the surface of the substrate with
isobutyl methacrylate polymer after the propelling of the droplets
of the dispersion of the synthetic construct of the structure F-S-
L.

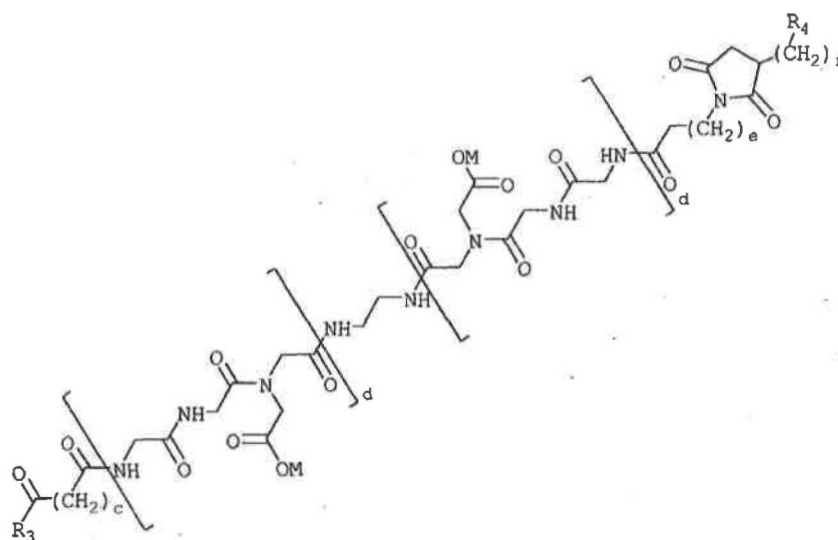
Preferably, when F is a glycan, S is selected from the group consisting of:



where:

- 5 a and b are independently the integer 3, 4 or 5; and
 R₁ and R₂ are, respectively, O of the glycan and N of the
 primary amino of a diacyl or dialkyl-glycerophospholipid or N
 of the primary amino of a diacyl or dialkyl-
 glycerophospholipid and O of the glycan, or

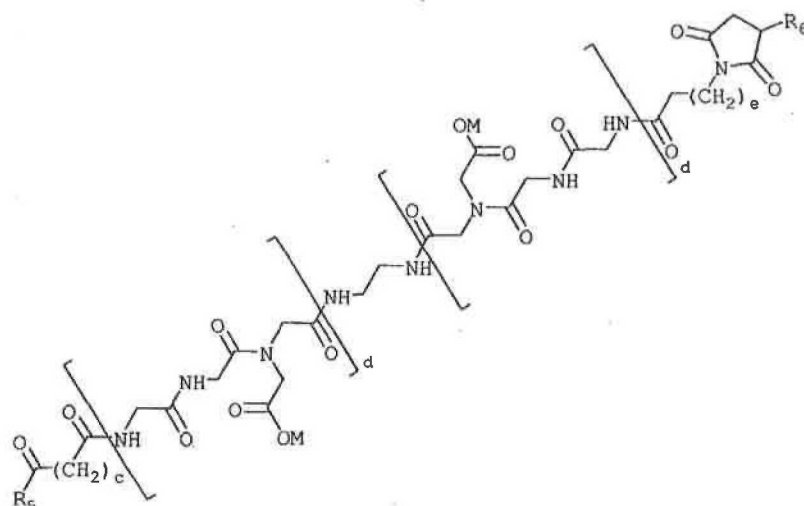
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where:

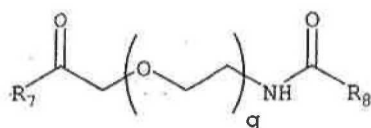
- M is CH₃ or H;
 c is the integer 3, 4 or 5;
 d and e are independently the integer 1 or 2;
 f is the integer 2, 3 or 4;
 R₃ is N of the primary amino of a diacyl or dialkyl-
 glycerophospholipid; and
 R₄ is O of the glycan.

Preferably, when F is a peptide, S is selected from the group consisting of:



where:

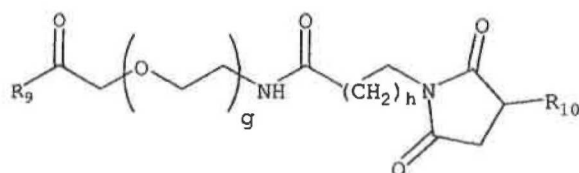
- 5 M is CH₃ or H;
 c is the integer 3, 4 or 5;
 d and e are independently the integer 1 or 2;
 R₅ is N of the primary amino of a diacyl or dialkyl-glycerophospholipid; and
 10 R₆ is S of the sulfhydryl of an amino acid residue of the peptide,



where:

- 15 g is a value in the range 6 to 14;
 R₇ and R₈ are, respectively, N of the amino terminus of the peptide and N of the primary amino of a diacyl or dialkyl-glycerophospholipid or N of the primary amino of a diacyl or dialkyl-glycerophospholipid and N of the amino terminus of the peptide,
 20 or

10



where:

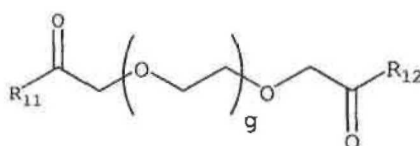
g is a value in the range 6 to 14;

h is the integer 1 or 2;

5 R₉ is N of the primary amino of a diacyl or dialkyl-glycerophospholipid; and

R₁₀ is S of the sulfhydryl of an amino acid residue of the peptide, or

or



10

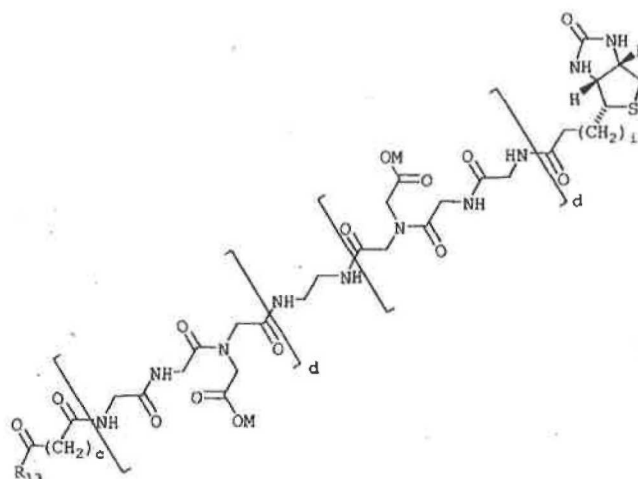
g is a value in the range 6 to 14;

R₁₁ and R₁₂ are, respectively, N of the amino terminus of the peptide and N of the primary amino of a diacyl or dialkyl-glycerophospholipid or N of the primary amino of a diacyl or dialkyl-glycerophospholipid and N of the amino terminus of the peptide.

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[followed by page 11]

Preferably, when F is a conjugator that is biotin, F-S is selected from the group consisting of:



where:

- 5 M is CH₃ or H;
 c is the integer 3, 4 or 5;
 d and e are independently the integer 1 or 2; and
 R₁₃ is N of the primary amino of a diacyl or dialkyl-
 glycerophospholipid.

10

F may or may not include an avidinylated functional moiety.

In a **second** aspect the invention provides a diagnostic test card or stick, microarray or multiwell plate fabricated using the method of the first aspect of the invention.

- 15 In the description and claims of this specification the following acronyms, terms and phrases have the meaning provided:

"Belt" means, with reference to inkjet printing, the means of attachment between the printhead and stepper motor.

- 20 "Control Circuitry" means, with reference to inkjet printing, that part of the printer that controls the mechanical aspects of operation of the printer.

"Dispersible in water" means a stable, single phase system is formed when the synthetic construct is contacted with water.

"Glycan" means a polysaccharide or oligosaccharide and includes the carbohydrate portion of a glycoconjugate, such as a
5 glycoprotein, glycolipid, or a proteoglycan.

"Immobilised" means covalently bound to a surface and "immobilising" and "immobilisation" have a corresponding meaning.

"Impact" means, with reference to printing on the surface of a substrate, a method of printing where an image is created by a
10 printer mechanism contacting the surface, e.g. character and dot matrix printers.

"Ink Cartridge" means with reference to inkjet printing, that part of the print head assembly comprising a reservoir for containing ink.

15 "Inkjet Printer" means a non-impact printer that propels droplets of ink onto the surface of a substrate to create an image consisting of a plurality of dots (typically between 45 and 65 μm in diameter).

"Localised" means associated with a surface by non-covalent
20 interactions and "localising" and "localisation" have a corresponding meaning.

"Microarray" means a two-dimensional array of small quantities of biological material.

"Monolithic" means, with reference to a printhead, the plurality
25 of orifices (nozzles) from which droplets of ink are propelled are formed in a single body of material, e.g. a silicon substrate, by means such as photolithography or chemical etching.

"Non-Impact" means, with reference to printing on the surface of a substrate, a method of printing where an image is created without
30 a printer mechanism contacting the surface, e.g. inkjet and laser printers.

"Picolitre" means a volume of 10^{-12} litre (pL).

"Piezoelectric" means, with reference to inkjet printing, the method of propelling droplets of ink from the orifices (nozzles) of the printhead by vibration of piezo crystals.

- 5 "Polar functional groups" means any one or more of a carbonyl (C=O), carboxyl (-COOH) or secondary amino (>NH) group.

"Printhead" means, with reference to inkjet printing, that part of the print head assembly comprising a plurality of orifices (nozzles) from which droplets of ink are propelled.

- 10 "Printhead Stepper Motor" means, with reference to inkjet printing, that part of the printer that drives the movement of the printhead across the surface of a substrate.

"Rollers" means, with reference to inkjet printing, a set of rollers operating to advance the surface of a substrate through

- 15 the transverse path of the printhead.

"Substrate Feed Stepper Motor" means, with reference to inkjet printing, that part of the printer that drives the rollers to advance the surface of the substrate through the transverse path of the printhead.

- 20 "Thermal Bubble" means, with reference to inkjet printing, the method of propelling droplets of ink from the orifices (nozzles) of the printhead by vaporizing a volume of the ink.

- "Spacer" means a chemical moiety distinct from the base (e.g. ethanolamine) of a glycerophospholipid comprising at least three
25 polar functional groups.

In the description and claims of this specification the amino acids of peptides are identified in accordance with Tables 1 to 4 of Annex C, Appendix 2 of the PCT Administrative Instructions (as in force from January 1, 2010).

- 30 The use of the terms "first", "second", "third", etc. with reference to elements, features or integers of the subject matter

defined in the Statement of Invention and Claims, or with reference to alternative embodiments or preferences of the invention is intended to distinguish between alternatives and is not intended to imply an order of preference unless specifically
5 stated.

The invention will now be described with reference to the following Table of Peptides, embodiments or examples, and the figures of the accompanying drawings pages.

BRIEF DESCRIPTION OF DRAWINGS

10 **Figure 1.** Schematic representation of a conventional inkjet printer adapted for use in a method of fabricating microarrays in accordance with the method of the invention.

Figure 2. Diagrammatic illustration of patterning of water dispersible synthetic constructs to provide test strips capable of identifying the
15 presence of a plurality of binding molecules in a test sample.

Figure 3. Diagrammatic illustration of patterning of water dispersible synthetic constructs to provide (A) test strips, and (B) multi-well microplates, capable of use in determining the titre a binding molecule in a test sample.

20 **Figure 4.** Appearance of samples of substrate to which solutions of the aminopropyl derivatives of blood group A (A_{tr1}) and blood group B (B_{tr1}) trisaccharides and the construct A_{tr1} -sp-Ad-DOPE (I) (FSL-A) had been applied following visualisation by: i) anisaldehyde (aluminium-backed silica gel plate); ii) immunostaining (aluminium-backed silica gel plate
25 with plasticizer); iii) immunostaining (aluminium backed C_{18} derivatised silica gel plate with plasticizer); iv) immunostaining (aluminium backed C_{18} derivatised silica gel without plasticizer); and v) immunostaining (nitrocellulose).

Figure 5. A multiwell plate fabricated according to the method described
30 showing visualisation of anti-A immunoglobulin binding to A_{tr1} -sp-Ad-DOPE (I) (FSL-A) applied at increasing densities (quantity per unit area) employing the standard ink cartridge and grey scale setting (30% "2" to 100% "9") of an EPSON Stylus™ Colour 460 printer.

Table of Peptides	SEQ ID NO:
<u>Cys</u> (Xaa) ₂ TrpThrProProArgAlaGlnIleThrGlyTyrLeuThrValGlyLeuThrArgArg	1
<u>Cys</u> (Xaa) ₂ TrpThrProProArgAlaGlnIleThrGlyTyrArgLeuThrValGlyLeuThrArgArg	2
<u>Cys</u> (Xaa) ₂ ValMetTyrAlaSerSerGly	3
ValMetTyrAlaSerSerGly(Xaa) ₂ <u>Cys</u>	4
AspTyrHisArgValMetTyrAlaSerSerGly(Xaa) ₂ <u>Cys</u>	5
ThrAsnGlyGluThrGlyGlnLeuValHisArgPhe(Xaa) ₂ <u>Cys</u>	6
ThrAsnGlyGluMetGlyGlnLeuValHisArgPhe(Xaa) ₂ <u>Cys</u>	7
AspThrTyrProAlaHisThrAlaAsnGluValSerGlu(Xaa) ₂ <u>Cys</u>	8
ThrTyrProAlaHisThrAlaAsnGluVal(Xaa) ₂ <u>Cys</u>	9
ProAlaHisThrAlaAsnGluVal(Xaa) ₂ <u>Cys</u>	10
TyrProAlaHisThrAlaAsnGlu(Xaa) ₂ <u>Cys</u>	11
ThrTyrProAlaHisThrAlaAsn(Xaa) ₂ <u>Cys</u>	12
ThrTyrProAlaHisThrAlaAsnGlu(Xaa) ₂ <u>Cys</u>	13
TyrProAlaHisThrAlaAsnGluVal(Xaa) ₂ <u>Cys</u>	14
ProAlaHisThrAlaAsnGluValSer(Xaa) ₂ <u>Cys</u>	15
AspThrTyrProAlaHisThrAlaAsnGlu(Xaa) ₂ <u>Cys</u>	16
TyrProAlaHisThrAlaAsnGluValSer(Xaa) ₂ <u>Cys</u>	17
SerGlnThrAsnAspLysHisLysArgAsp(Xaa) ₂ <u>Cys</u>	18
GlnThrAsnAspLysHisLysArgAspThrTyr(Xaa) ₂ <u>Cys</u>	19

Table of Peptides	SEQ ID NO:
GlnThrAsnAspLysHisLysArgAspThrTyrSerSerGlnThrAsnAspMetHisLysArgAspThrTyr (Xaa) ₂ Cys	20
GlnThrAsnAspMetHisLysArgAspThrTyr (Xaa) ₂ Cys	21
SerSerGlnThrAsnAspLysHisLysArg (Xaa) ₂ Cys	22
SerSerGlnThrAsnAspLysHisLysArgAspThrTyr (Xaa) ₂ Cys	23
SerSerGlnThrAsnAspMetHisLysArgAspThrTyr (Xaa) ₂ Cys	24
SerSerGlnThrAsnAspLysHisLysArgAspThrTyrSerSerGlnThrAsnAspMetHisLysArgAspThrTyr (Xaa) ₂ Cys	25
GlnThrAsnAspLysHisLysArgAspThr (Xaa) ₂ Cys	26
SerGlnThrAsnAspLysHisLysArgAspThr (Xaa) ₂ Cys	27
ThrAsnAspLysHisLysArgAspThrTyrPro (Xaa) ₂ Cys	28
GluGluThrGlyGluThrGlyGlnLeuVal (Xaa) ₂ Cys	29
GluGluGluThrGlyGluThrGlyGlnLeu (Xaa) ₂ Cys	30
GluThrGlyGluThrGlyGlnLeuValHis (Xaa) ₂ Cys	31
SerProProArgArgAlaArgValThr (Xaa) ₂ Cys	32
TyrArgTyrArgTyrThrProLysGluLysThrGlyProMetLysGlu (Xaa) ₂ Cys	33
TrpGlnProProArgAlaArgIle (Xaa) ₂ Cys	34
ThrIleThrGlyLeuGluProGlyThrGlu (Xaa) ₂ Cys	35

Figure 6. Template design for use in the fabrication of a multiwell plate for use in quantifying antibody titres.

Figure 7A. A fabricated multiwell plate employing the template of Figure 6.

- 5 **Figure 7B.** An enlargement of one of the wells of the fabricated multiwell plate of Figure 7A.

Figure 8A. A fabricated multiwell plate employing a template to identify the location of each well.

- 10 **Figure 8B.** An enlargement of one of the wells of the fabricated multiwell plate of Figure 8A.

Figure 9. The fabricated multiwell plates used in the detection of binding molecules in biological samples.

- Figure 10.** Immunostaining with monoclonal antibody of the surface of substrates (silica gel and paper) printed with a dispersion of FSL-A. The identity of the substrate employed is identified by the words appearing following immunostaining: silica gel (A), Sapphire Cast Coated (Spicers) (B), Impress Silk (Spicers) (C), Impress Gloss (Spicers) (D), Hello Silk (Spicers) (E), Black Velvet Artboard (F), G-Print Matt (G), Alpine Artboard (H), Superfine Hi Gloss (Spicers) (I and J) and uncoated paper (K).
- 15
20

- Figure 11.** Immunostaining with monoclonal and polyclonal (serum) antibody of the surface of substrates (paper) printed with a dispersion of FSL-A. The identity of the substrate employed is identified by the words appearing following immunostaining: Sapphire Cast Coated (Spicers) (B), Impress Silk (Spicers) (C), Impress Gloss (Spicers) (D), G-Print Matt (G), Superfine Hi Gloss (Spicers) (J) and uncoated paper (K).
- 25

- Figures 12A and 12B.** Immunostaining with alkaline phosphatase conjugated streptavidin of the surface of substrates (nitrocellulose and paper) printed with a dispersion of FSL-Biotin. The identity of the substrate employed is identified by the words appearing following immunostaining: Sapphire Cast Impress Gloss (Spicers) (D), G-Print Matt (G), Superfine Hi Gloss (Spicers) (J), uncoated paper (K) and nitrocellulose (L).
- 30

- Figure 13.** The structure of the construct A_{tr1}-sp-Ad-DOPE (I) (FSL-A) printed on paper using a dispersion of the construct according to the method of the invention.
- 35

DETAILED DESCRIPTION

The advantages provided by the invention arise from the favourable working interrelationship between a combination of features.

5 Firstly, the synthetic constructs of the structure F-S-L are readily dispersible in water ("water soluble" as defined herein). Secondly, the synthetic constructs remain localised to the surface of a substrate despite washing with aqueous solutions. Thirdly, inkjet printer technology has proven to be readily adaptable as a means of applying the dispersions of the synthetic constructs to
10 the surface of the substrate.

Adopting the analogy with conventional inkjet printing the dispersions of synthetic constructs are used as an "ink" to print on the surface of a substrate used as "paper". Indeed it has been discovered that the synthetic constructs are localised to the
15 surface of paper with sufficient strength that the functional moiety is not washed away during blocking and washing steps routinely used in diagnostic assays. The use of existing inkjet printer technology permits the numbers of functional moieties to be localised to the surface of the substrate with greater control
20 and accuracy. The ability to accurately control both the quantity and location of functional moieties localised to the surface of a substrate also permits the printing of "images" that improve the accuracy and reliability of assay results.

The preparation of water dispersible synthetic constructs F-S-L
25 with a range of functional moieties (F) including biotin, glycans and peptides is described in the specifications accompanying international application nos. PCT/NZ2005/000052 (publ. no. WO2005/090368), PCT/NZ2006/000245 (publ. no. WO2007/035116) and PCT/NZ2008/000266 (publ. no. WO 2009/048343).

30 The selection of a spacer (S) provides a synthetic construct that is readily dispersible in water. It is also apparent that "printed" synthetic constructs are oriented to permit interaction between the functional moiety (F) with a putative binding molecule.

The method of the invention provides the advantage that the requirement for subsequent blocking of unreacted groups on a chemically activated surface (cf. chemical immobilisation) is negated. An additional advantage is the prospect of eluting
5 binding molecule bound to its target functional moiety from the surface by the use of solvents. The opportunity to then characterise the functional moiety and binding molecule arises.

The use of the chemistry employed in the manufacture of conventional reverse phase media such as C₈, C₁₈, etc. was
10 initially considered to be most appropriate for the preparation of lipophilic surfaces to which the constructs could be localised. In this context it should be noted that the term "lipophilic" is being used to encompass any chemistry that provides a surface with a strong affinity for the lipid (L) of the synthetic construct.
15 It is to be recognised that some substrates provide a lipophilic surface without the requirement for chemical modification, e.g. nitrocellulose (Fukui *et al* (2005)) and polystyrene (Huang *et al* (2006)). The term "lipophilic" as used herein is to be understood as a functional feature. Of particular note in the context of the
20 present invention is that both coated and uncoated printer paper have been demonstrated to provide a suitable "lipophilic" surface.

The monomeric dissociation constant (K_D) in a carbohydrate-protein interaction is typically in the millimolar (mM) range. Carbohydrate mediated biological responses often occur through
25 multivalent interactions on the cell surface in order to achieve high affinity and specificity (Chung-Yi *et al* (2009)). It is anticipated that localising the functional moieties to the surface of a substrate by the interaction of the lipophilic surface and the lipid moiety of the synthetic construct F-S-L permits the
30 functional moieties of a population of deposited synthetic constructs to have a greater opportunity to participate in multivalent interactions with binding molecules, e.g. glycan binding proteins (GBPs).

The adaptation of existing inkjet printing technology to deposit
35 quantities of a dispersion of synthetic construct provides a

convenient and cost effective means of fabricating diagnostic test cards and sticks, microarrays and multiwell plates of standard dimensions. Indeed it will be recognised by analogy with conventional colour inkjet printing that the patterning of deposition is also readily achievable. Chambers containing dispersions of populations of synthetic construct are substituted for the colour cartridges of the inkjet printer. Chamber size and design can be readily optimised for the fabrication of microarrays and use of aqueous dispersions. The inclusion of a relatively volatile solvent in the aqueous dispersion is anticipated to facilitate fabrication of the microarrays by promoting evaporation of the vehicle. However, as conventional inject technology permits the delivery of droplets of small size the surface area to volume ratio results in a sufficient rate of evaporation to permit the use of water as a vehicle for the dispersions.

Printheads of designs adaptable for use in the method of the present invention are well described. A description of the adaptation of an inkjet printer for use in the fabrication of microarrays in accordance with the method of the invention will now be described with reference to Figure 1 of the accompanying drawings.

Figure 1 is a side cross-sectional view schematically showing the printhead (1) of an inkjet printer and the surface of a substrate (2) in juxtaposition. The printhead (1) is mounted on a carriage (3) that permits reciprocating motion (4) of the printhead (1) relative to the surface of the substrate (2).

The printhead (1) comprises a plurality of modules (4a, 4b, 4c) comprising chambers (5a, 5b, 5c), each containing a dispersion of a population of synthetic construct F-S-L. Each chamber (5a, 5b, 5c) includes an orifice (nozzle) (6a, 6b, 6c) through which a droplet of the dispersion is discharged when a voltage is applied to a piezoelectric assembly (7a, 7b, 7c) in fluid communication with the dispersion.

Each chamber (5a, 5b, 5c) additionally includes a sealable port (8a, 8b, 8c) through which the contents of the chamber may be replenished and a convoluted channel (9a, 9b, 9c) to provide for pressure equalisation subsequent to the discharge of a droplet.

- 5 The application of a voltage to each of the piezoelectric assemblies (7a, 7b, 7c) is under the control of a controller (10). In turn the controller and reciprocating motion of the printhead relative to the surface of the substrate are under computer control to permit patterning of the surface of the substrate (2).
- 10 The application by the controller (10) of a voltage to the piezoelectric assembly (7a) causes a droplet of predetermined size to be discharged via the orifice (6a) with sufficient momentum to traverse the distance to the juxtaposed surface (2).

- On contact with the lipophilic surface it is anticipated the synthetic constructs F-S-L will orient so that the lipid moiety (L) is associated with the surface. This dynamic process is promoted by evaporation of the aqueous vehicle and will be dependent on ambient conditions of temperature and pressure as well as droplet volume as well as the percentage, if any, of co-
- 15 solvent, e.g. methanol, present in the aqueous vehicle.
- 20

- It will be apparent from the foregoing description that droplets consisting of different populations of synthetic construct may be deposited in the same discrete area on the surface of the substrate. Microarrays with patterning of this type may be of
- 25 assistance in identifying binding molecules that form multivalent interactions with a plurality of receptors present in the glycocalyx of cells.

- Similarly it will be apparent from the foregoing description that droplets consisting of different populations of synthetic
- 30 construct may be deposited in the discrete areas spaced apart on the surface of the substrate and at different concentrations. Microarrays with patterning of this type may be of assistance in identifying the avidity and specificity of binding molecules.

As discussed above the non-covalent localisation of functional moieties to a surface presents the prospect of eluting the binding molecule bound to its target functional moiety from the surface. The opportunity to characterise the functional moiety and binding molecule then arises.

In anticipated embodiments the method is used to conveniently and cost effectively produce diagnostic test cards and strips and arrays of the type illustrated diagrammatically in Figure 2 and Figure 3.

10 The diagnostic test cards and strips and arrays may be produced in large numbers with a high degree of reproducibility making them eminently suitable for use in inter-laboratory standardisation.

Reliability and ease of use also suggests the use of the diagnostic test cards and strips and arrays in over-the-counter home test kits.

The patterning of the different populations of synthetic construct may be readily adjusted to correspond to the format of the automated reading device where one is to be used.

In use a test sample is contacted with the diagnostic test strip or array for a predetermined time to allow binding of binding molecules present in the test sample to bind to the functional moiety, e.g. glycotope, of the deposited synthetic construct.

The surface of the diagnostic test strip or array is then washed with an aqueous buffer, such washing facilitated by the lipophilicity of the surface of the test strip or array.

The presence of bound binding molecule may then be detected by use of detection systems such as anti-IgG enzyme conjugates that give rise to a chromogenic response in the presence of the appropriate reagents.

30 It will be recognised that the ability to deposit populations of synthetic constructs in discreet areas conveniently provides a negative control for the assay to be performed.

By way of illustration, if the assay to be performed is a detection of a particular binding molecule in a sample fluid by chromogenic means the non-deposited area in contact with the sample fluid provides the negative control.

- 5 Confirmation of the presence of the binding molecule in the sample fluid is provided by the contrast in chromogenic response between the deposited and non-deposited (negative control) area.

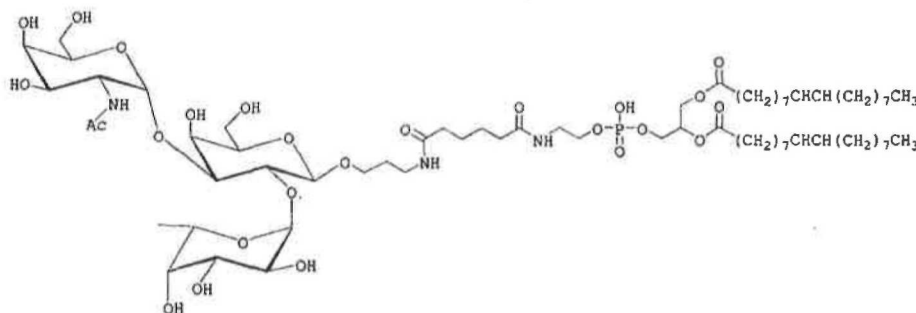
- Similarly, it will be recognised that a positive control may be incorporated by depositing in a discreet area a population of
10 synthetic construct comprising a functional moiety (F) known to be present at detectable levels in all sample fluids to be tested.

Assurance that the assay has been performed correctly is provided by the contrast in chromogenic response between the deposited (positive control) and non-deposited area.

- 15 The discreet area in which the synthetic construct providing for this positive control is deposited may be delineated so as to provide a confirmatory indication to the user, such as a tick symbol or smiley face, or readable phrase.

EXAMPLES

- 20 Dispersions of the aminopropyl derivatives of blood group A trisaccharide ($A_{tri}-S_1$) and blood group B trisaccharide ($B_{tri}-S_1$) were prepared at a concentration of 0.6 mM in phosphate buffered saline (pH 7.2) (PBS). A solution of the construct $A_{tri}-sp-Ad-DOPE$ (FSL-A) was also prepared at a concentration of 0.6 mM in PBS.



25

FSL-A

The solutions were applied using a fine tipped artist's paintbrush onto the surface of each of three substrates:

1. Aluminium-backed silica gel thin layer chromatography plates (Alugram Nano-SIL G silica TLC plate, 0.2 nm Nano silica gel 5 60, Macherey-Nagel);
2. Aluminium-backed C₁₈ derivatised silica gel plates; and
3. Nitrocellulose membranes.

One of the samples of aluminium-backed silica gel thin layer chromatography plates to which the solution had been applied was 10 sprayed with a solution of anisaldehyde. The sprayed plate was heated to 200 °C to visualise staining.

The remaining samples of aluminium-backed silica gel thin layer chromatography plates and aluminium-backed C₁₈ derivatised silica gel thin layer chromatography plates to which the solution had 15 been applied were immersed in a solution of PLEXIGUM™ P28 (0.5% isobutyl methacrylate polymer in n-hexane and diethyl ether) for 1 minute and then air dried.

The surface of all samples to which the solutions had been applied were then immersed in a solution of 2% (w/v) bovine serum albumin 20 (BSA) in PBS prior to being flooded with a dilution of anti-A immunoglobulin (EPICLONE™ monoclonal, CSL Limited).

The flooded surfaces of the substrates were then washed with PBS prior to being flooded with a 1:400 dilution of alkaline phosphatase conjugated sheep anti-mouse immunoglobulin (Chemicon) 25 for 30 minutes. The flooded surfaces of the substrates were then washed with PBS followed by a washing of substrate buffer (100 mM Tris, 100 mM NaCl, 50 mM MgCl₂, pH 9.5).

The substrate buffer washed samples were then flooded with a 1:55 dilution of chromogenic substrate (18.75 mg/mL nitro blue 30 tetrazolium chloride and 9.4 mg/mL 5-bromo-4-chloro-3-indolyl phosphate, toluidine salt) (NBTC-BCIP) for 15 minutes. The appearance of the samples following incubation with substrate is provided in Figure 4.

Fabrication of multiwell plates

- Thirty two holes of 7 mm diameter were cut in a 85 x 63 x 3 mm planar piece of acrylic in a 4 x 8 matrix so as to correspond with the positions of half of the wells of a standard multiwell
- 5 microplate. The upper surface of the planar piece of acrylic was also engraved with letters along the long edge and numbers along the short edge so as to allow each hole in the matrix to be uniquely identified by a two character alphanumeric code. Employing the same template used to direct laser cutting of the
- 10 planar piece of acrylic, a solution of the construct A_{TE1}-S₁-Ad-DOPE (FSL-A) at a concentration of 1 mg/mL in water was printed onto the surface of an aluminium-backed silica gel plate.

- The solution was loaded into the ink cartridge of an EPSON STYLUS™ Colour 460 piezoelectric inkjet printer. A concentration of 0.1%
- 15 (w/v) bromophenol blue was included to assist in visualising the printed area. The numerals "2" to "9" were printed using the same solution applied at increasing densities (quantity per unit area) corresponding to the grey scale settings of the printer. The alphanumeric character "2" was printed at a grey scale setting of
- 20 30% through to the alphanumeric character "9" printed at a grey scale setting of 100%. The printed plate was washed by placing in a beaker of deionised water for a period of 20 minutes and then air dried. The air dried printed plate was then immersed in PLEXIGUM™ P28 for a period of 1 minute before being air dried for
- 25 a second time. The laser cut planar piece of acrylic was then adhered to the surface of the aluminium-backed painted silica gel plate using multipurpose adhesive. A fabricated 4 x 8 well microplate was thereby prepared.

Detection of binding molecule

- 30 A 100 µL volume of a 2% (w/v) solution of BSA in PBS was dispensed into each well of the fabricated microplate. The plates were incubated for 30 minutes before aspirating the solution of BSA from each well and rinsing. A 100 µL volume of a 1:4 dilution of mouse anti-A immunoglobulin was then dispensed into each well and

the plate incubated for 30 minutes before rinsing each well with PBS. A 100 μ L volume of a 1:400 dilution of anti-mouse immunoglobulin was then dispensed into each well and the plate incubated for 30 minutes before washing each well with substrate
5 buffer. A 100 μ L volume of a 1:55 dilution of chromogenic substrate was then dispensed into each well and the plates incubated for 50 minutes. Each well was finally washed with deionised water and the plate air dried. The air dried plate is presented in Figure 5.

- 10 The method of fabricating described provides for the convenient manufacture of multiwell plates for simultaneous qualitative and quantitative assessment of binding molecules.

Template design

- 15 Different designs of templates for use in the microarray formats can conveniently made in accordance with user requirements employing standard word processing or drawing software packages as illustrated in Figures 2, 3 and 5 to 9 of the drawings pages.

Quantitative antibody testing

- 20 By way of illustration of a template design Figure 6 provides the design of a template for use in quantifying antibody titres. The template is dimensioned to correspond to the dimensions of a standard multiwell microplate. The fabricated multiwell plate may therefore be handled by existing dispensers, washers and camera-based readers of multiwell microplates.

- 25 A well of the template corresponds to two wells of a standard multiwell microplate. The two wells on the right of the template may be employed as control wells. The alphanumeric characters and other symbols shown in the template are engraved in the acrylic and the wells are cut out using a laser. The piece of planar
30 acrylic produced is then adhered to a printed silica gel plate.

Solutions of constructs F-S-L are printed employing the same template in which the bars of increasing colour density correspond

to increasing densities (quantity per unit area) of construct applied. It will be recognised that the standard gray scale settings of the printer may be employed to provide these increasing densities.

- 5 In the template the printing of two constructs is presented. The constructs F-S-L each comprise a different ligand (F) for one or more binding proteins. The binding specificity of one or more binding proteins in the sample may therefore be conveniently assessed.
- 10 In the template the two constructs are printed as bars of decreasing density and increasing length.

By the use of the template (and others of comparable design) quantitative assessments of binding may be made in a single well of a multiwell microplate. An internal control (background
15 signal) is provided by the unprinted region outside the discrete area on the surface of the substrate to which the constructs have been applied.

- The design of the template also permits monitoring of assays and a convenient means to identify the optimum time to terminate
20 incubations. The controls wells (circled in Figure 6) have only one of the two construct per well, but printed to provide ladders of increasing density going in both directions. When the middle band appears and a whole line across the well is visible the optimum time to terminate the incubation is indicated.
- 25 A fabricated microwell plate employing the design of template illustrated in Figure 6 and used to determine the titre of antibody according to the method described above is illustrated in Figures 7A and 7B.

Well identification

- 30 By way of further illustration in circumstances where unequivocal identification of a well in which a positive reaction has occurred is required, a template of the design incorporated into the

fabricated multiwell plate illustrated in Figures 8A and 8B may be employed.

The alphanumeric combination of characters that appear in wells in which a positive reaction has occurred, unequivocally identify the well without reliance on the user correctly determining the coordinates of the well location. The likelihood of user error in manual operations is thereby greatly reduced.

Detection of binding molecule in a biological sample

To confirm the utility of the fabricated microwell plate in the detection of binding molecules in biological samples the production of anti-A antibody in the serum of mice was elicited by immunisation with A substance saliva. Individual mice were immunised 2, 3 or 4 times with A substance saliva over a three week period. Naïve mice having had no immunisation were used as a control. Elicitation of anti-A antibody in the sera of immunised mice was confirmed by transfusion of modified red blood cells ("kodecytes") according to the methods described in international application no. PCT/NZ2009/000209. A 32 well microtiter plate was fabricated according to the method described above using a design of template in which the alphanumeric symbol "A" was printed in a location corresponding to the base of each well. Each well was filled with 2% BSA in PBS and incubated at room temperature for at least one hour. Samples of sera collected from immunised mice were diluted 4-fold in 2% BSA in PBS. The 2% BSA in PBS was removed from the wells following incubation and the diluted samples of sera introduced into individual wells using a pipette. The plates were then incubated at room temperature for at least 90 minutes. Following incubation the samples were removed from each well and the plate washed several times with PBS. Excess PBS was removed by blotting of the surface of the plate and each well then filled with a 400-fold dilution of mouse anti-Ig antibody conjugated with alkaline phosphatase. The plates were then incubated for an hour before removing the antibody conjugate solution and washing several times with PBS. The plate was then washed several times with substrate buffer before filling each

well of the plate with the chromogenic substrate NBTC-BCIP. The plates were then incubated for 15 to 20 minutes until the printed alphanumeric character appeared. The substrate was then removed and the plate washed under a gentle stream of deionised water and dried. The wells to which anti-A antibody containing samples were introduced were clearly identifiable as illustrated in Figure 9. It will be recognised that alternative template designs may be developed for the assessment of binding to multiple constructs and the required multiwell microplates conveniently fabricated according to the method described.

Printing of paper

The ability of standard printing papers to serve as the substrate for use in the method of the invention was evaluated. Solutions of the construct A_{tri} -sp-Ad-DOPE (FSL-A) and a solution of the aminopropyl derivative of the A trisaccharide (A_{tri} -sp-NH₂) were printed on to various types of commercially available printing papers as previously described. An ink jet printer (EPSON STYLUS™ T21) with refillable cartridges modified to hold a smaller volume was employed. The construct A_{tri} -sp-Ad-DOPE (FSL-A) and the aminopropyl derivative of the A trisaccharide (A_{tri} -sp-NH₂) were prepared as solutions at a concentration of 6 mM. Each one of the solutions was used to fill separate modified cartridges permitting both solutions to be printed at the same time on the same sample of paper. To facilitate identification and as an illustration of one of the advantages of the invention the identification of the solution and trade name of the paper employed as the sample were printed. Following printing of the two solutions each sample of paper was blocked with a 2% (w/v) solution of BSA and immunostained with monoclonal anti-A and then anti-mouse IgG conjugated to alkaline phosphatase and the chromogenic substrate NBT-BCIP. The immunostained samples of printed paper are presented in Figure 10. It will be observed that there was no immunostaining of the sample of printed paper in the region where the aminopropyl derivative of the A trisaccharide (A_{tri} -sp-NH₂) was printed. It is assumed that the aminopropyl derivative of the A

trisaccharide ($A_{tri-sp-NH_2}$) was washed away during the blocking step and immunostaining procedure. Although the majority of the papers employed in the study were coated papers it was also demonstrated that normal uncoated paper could also serve as a suitable
5 substrate.

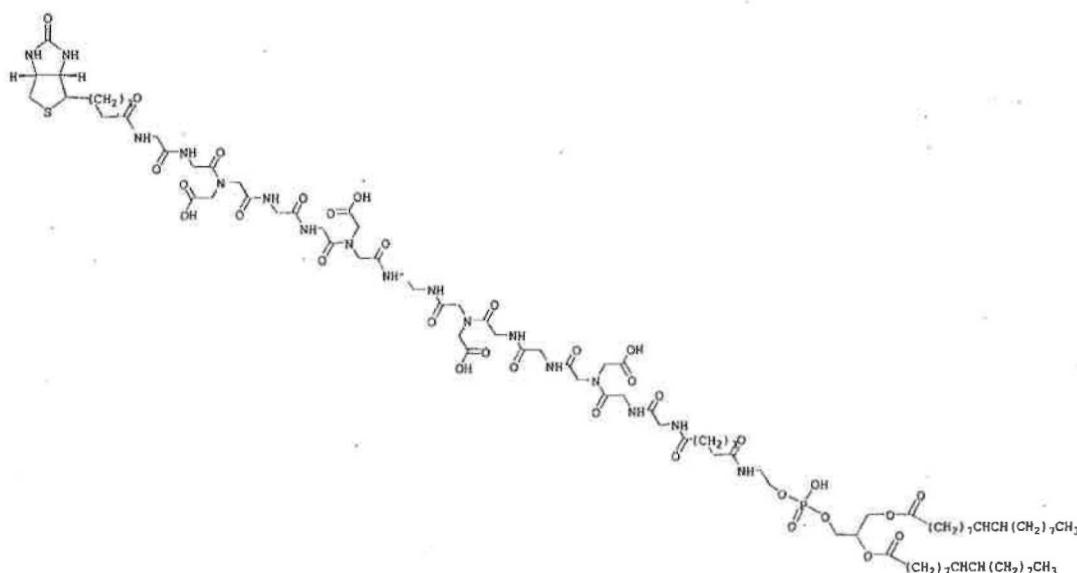
Biological sample

Samples of printed papers were prepared as described under the preceding heading. On this occasion, polyclonal human blood group O serum and mouse anti-human Ig were employed in the
10 immunostaining procedure. A concentration of 0.05% w/v bromophenol blue was included in the solutions of the construct $A_{tri-sp-Ad-DOPE}$ (FSL-A) to permit visualisation of the printed solutions. The dye was removed following the initial step of printing by placing the samples of printed paper in a beaker of deionised water for 15
15 minutes followed by air drying. The dried printed samples of paper were then blocked with a solution of BSA for 30 minutes as previously described. The surface of each sample of printed paper was then flooded with a 1 in 4 dilution of either anti-A monoclonal antibody or the O serum for 60 minutes at room
20 temperature. The flooded surface of the printed sample of paper was then washed repeatedly by flooding the surface of each sample of printed paper with PBS for 20 seconds and rinsing with PBS. A comparison of the appearance of the printed samples of paper at each step of the procedure is presented in Figure 11. It will be
25 observed that no immunostaining in the region where the aminopropyl derivative of the A trisaccharide ($A_{tri-sp-NH_2}$) was printed occurs. The construct $A_{tri-sp-Ad-DOPE}$ (**I**) (FSL-A) is detected following immunostaining with either monoclonal or polyclonal (serum) antibodies. Certain of the coated papers were
30 observed to provide great contrast in the immunostaining procedure when polyclonal (serum) antibodies were employed.

Printing of FSL-Biotin

A dispersion of the construct FSL-Biotin at a concentration of 1 mg/ml (6 mM) in PBS and containing 0.05% (w/v) bromophenol blue

- was prepared. A volume of the solution was injected into the modified refillable cartridge of a piezoelectric printer (EPSON™ Stylus T21). The dispersion of the construct FSL-Biotin was printed onto samples of coated papers and uncoated paper. The
- 5 dispersion of the construct FSL-Biotin was also printed onto aluminium-backed silica TLC plates (0.2mm Nano silica gel 60, Macherey-Nagel) and nitrocellulose membranes (0.02 µL pore size, Invitrogen).



FSL-Biotin

- The samples of printed paper were placed in a beaker of deionised water for 15 minutes to remove the bromophenol blue dye and then air dried. The sample of aluminium backed silica gel TLC plate was
- 15 immersed in a solution of 0.5% (w/v) polyisobutylmethacrylate in n-hexane and diethyl ether (PLEXIGUM P28) for one minute and then air dried. Neither the samples of printed paper nor sample of printed nitrocellulose was subjected to this treatment prior to immunostaining. For immunostaining the samples were first blocked
- 20 by flooding the surface with a solution of 2% (w/v) BSA in PBS. The surface of each sample was then flooded with streptavidan-alkaline phosphatase conjugate (sigma) at a concentration of 2

µg/ml for 30 minutes at room temperature. The samples were then washed with PBS by flooding the surface of the membranes with PBS for 20 seconds and repeating 6 times with fresh PBS for each washing step. The printed samples were then washed with substrate
5 buffer (100 mM Tris, 100 mM NaCl, 50 mM MgCl₂, pH 9.5) prior to flooding the surface of each sample with NBT/BCIP substrate (18.75 mg/ml nitro blue tetrazolium chloride and 9.4 mg/ml 5-bromo-4-chloro-3-indoyl-phosphate, toluidine salt in 67% (v/v) DMSO (Roche) diluted 50-fold in substrate buffer and incubated for 50
10 minutes at room temperature. The printed samples were rinsed with deionised water to stop the chromogenic reaction. The results of immunostaining each of the samples are presented in Figures 12A and 12B.

Although the invention has been described by way of exemplary
15 embodiments it should be appreciated that variations and modifications may be made without departing from the scope of the invention. Where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in this specification.

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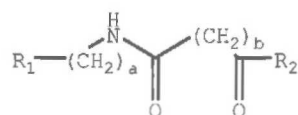
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CLAIMS

- 1) A **method** of localising a functional moiety (F) to at least one discrete area on a surface of a substrate including the step of propelling droplets of a dispersion of a synthetic construct of the structure F-S-L from a plurality of orifices located in a monolithic print head onto the surface of the substrate where S is a spacer selected to provide a construct that is dispersible in water in the absence of organic solvents or detergents at a temperature of 25 °C and L is a diacyl- or dialkyl-glycerophospholipid.
- 2) The method of claim 1 where the at least one discrete area is in the shape of a symbol.
- 3) The method of claim 1 where the at least one discrete area is a pattern comprising a combination of indicia in which the dispersion of the synthetic construct is present at different densities.
- 4) The method of any one of claims 1 to 3 where the propelling droplets from a plurality of orifices is from a plurality of orifices located in the monolithic print head of an inkjet printer.
- 5) The method of claim 4 where the volume of each of the droplets is 1 to 100 picolitres (pL).
- 6) The method of claim 5 where the volume of each of the droplets is 1 to 50 pL.
- 7) The method of claim 6 where the volume of each of the droplets is 1 to 5 pL.
- 8) The method of any one of claims 1 to 7 where the concentration of the synthetic construct in the dispersion is 1 μ molar (μ M) to 10 mmolar (mM).
- 9) The method of claim 8 where the concentration of the synthetic construct in the dispersion is 10 μ M to 10 mM.
- 10) The method of claim 9 where the concentration of the synthetic construct in the dispersion is 0.1 to 10 mM.
- 11) The method of any one of claims 1 to 10 where L is a phosphatidylethanolamine.

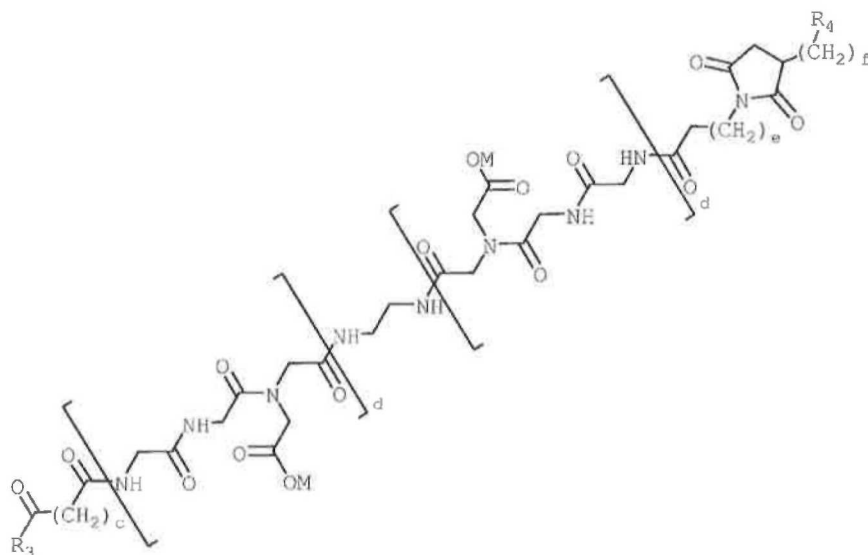
- 12) The method of claim 11 where L is 1,2-O-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) or 1,2-O-distearyl-sn-glycero-3-phosphatidylethanolamine (DSPE).
- 13) The method of claim 11 or 12 where F is an oligosaccharide.
- 14) The method of claim 11 or 12 where F is an oligopeptide.
- 15) The method of claim 11 or 12 where F is biotin.
- 16) The method of claim 13 where S is:

(i)



where a and b are independently the integer 3, 4 or 5; and R₁ and R₂ are, respectively, O of the oligosaccharide and N of the primary amino of the phosphatidylethanolamine or N of the primary amino of the phosphatidylethanolamine and O of the oligosaccharide, or

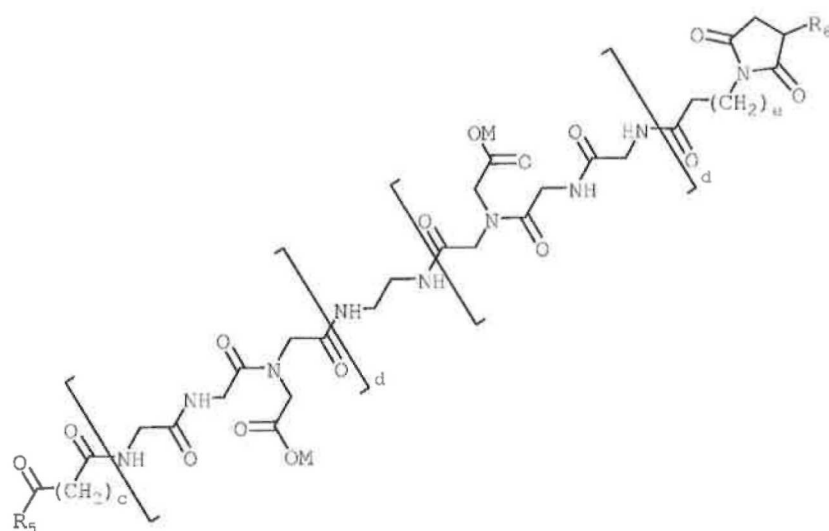
(ii)



where M is CH₃ or H; c is the integer 3, 4 or 5; d and e are independently the integer 1 or 2; f is the integer 2, 3 or 4; R₃ is N of the primary amino of the phosphatidylethanolamine; and R₄ is O of the oligosaccharide.

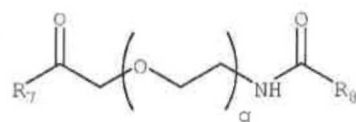
17) The method of claim 14 where S is:

(i)



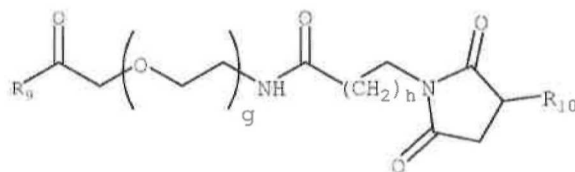
where M is CH₃ or H; c is the integer 3, 4 or 5; d and e are independently the integer 1 or 2; R₅ is N of the primary amino of the phosphatidylethanolamine; and R₆ is S of the sulfhydryl of an amino acid residue of the oligopeptide,

(ii)



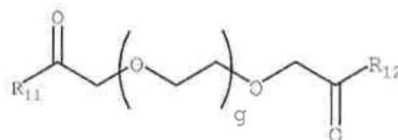
where g is a value in the range 6 to 14; R₇ and R₈ are, respectively, N of the amino terminus of the oligopeptide and N of the primary amino of the phosphatidylethanolamine, or N of the primary amino of the phosphatidylethanolamine and N of the amino terminus of the oligopeptide,

(iii)



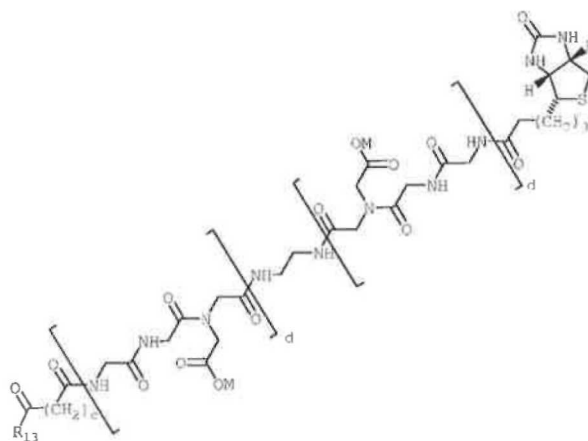
where g is a value in the range 6 to 14; h is the integer 1 or 2; R_9 is N of the primary amino of the phosphatidylethanolamine; and R_{10} is S of the sulfhydryl of an amino acid residue of the oligopeptide, or

(iv)



where g is a value in the range 6 to 14; R_{11} and R_{12} are, respectively, N of the amino terminus of the oligopeptide and N of the primary amino of the phosphatidylethanolamine, or N of the primary amino of the phosphatidylethanolamine and N of the amino terminus of the oligopeptide.

18) The method of claim 15 where F-S is:



where M is CH_3 or H ; c is the integer 3, 4 or 5; d and e are independently the integer 1 or 2; and R_{13} is N of the primary amino of the phosphatidylethanolamine.

19) A **diagnostic test card or stick, microarray or multiwell plate** fabricated using the method of any one of claims 1 to 18.