Specific Recognition of Lectins by Oligonucleotide Glycoconjugates and Sorting on a DNA Microarray

Jing Zhang, Gwladys Pourceau, Albert Meyer, Sébastien Vidal, Jean-Pierre Praly, Eliane Souteyrand, Jean-Jacques Vasseur, François Morvan and Yann Chevolot**

Supporting Information

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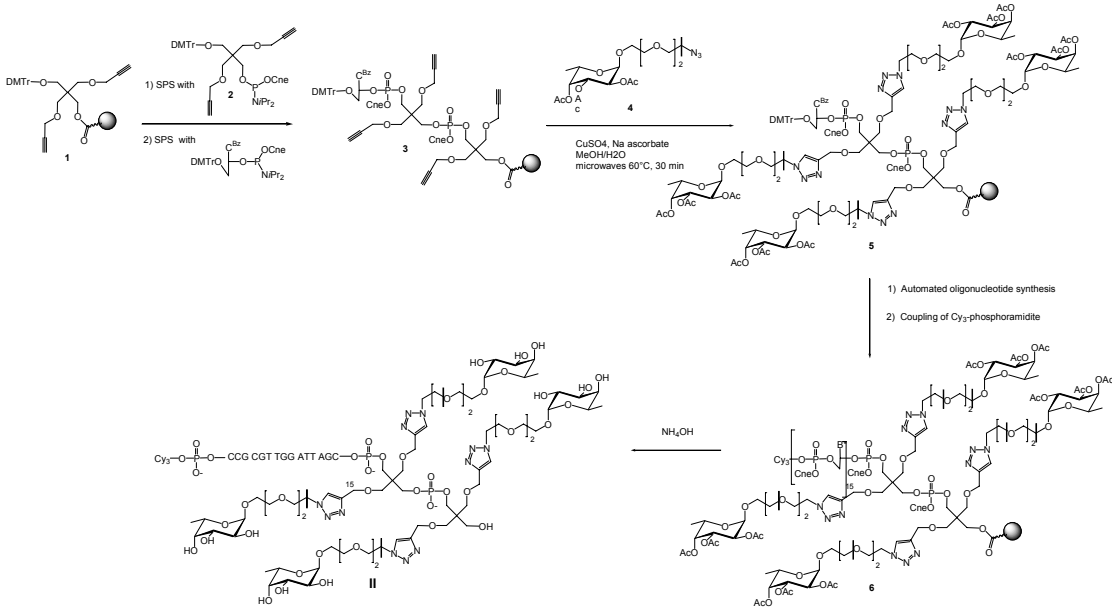
1 Synthesis of Glycoconjugates I and II.

Synthesis of trigalactosyl oligonucleotide conjugate **I** is described in reference [1] and the tetrafucosyl oligonucleotide was synthesized according to scheme S1



Scheme S1: Synthesis of the tetra-fucose glycomimetic **II**. Solid phase synthesis (SPS)

Synthesis of tetrafucosyl oligonucleotide conjugate II.

Solid-supported tetra-propargyl scaffold **3:** The solid-supported tetra-propargyl scaffold **3** was synthesized at a 1-μmole scale on a DNA synthesizer (ABI 394), using standard phosphoramidite chemistry, on bis-propargyl solid support **1**[2] using bis-propargyl phosphoramidite **2**[3] (0.09 M in anhydrous CH_3CN) and commercially-available cytidine phosphoramidite (0.075 M in anhydrous CH_3CN). Detritylation was performed with 2.5% DCA in CH_2Cl_2 for 35 s. Benzylmercaptotetrazole was used as the activator (0.3 M in anhydrous CH_3CN). The capping step was performed with acetic anhydride, using a commercial solution (Cap A: Ac_2O, pyridine, THF 10:10:80 v/v/v and Cap B: 10% *N*-methylimidazole in THF)

for 15 s. Oxidation was performed with a commercial solution of iodide (0.1 M I_2, THF, pyridine/water 90:5:5, v/v/v) for 13 s.

Procedure for Cu(I)-catalyzed 1,3-dipolar cycloaddition: To the solid-supported tetra-propargyl scaffold **3** (1 μmol) were added protected *O*-2,3,4- tri acetyl fucosyl azide **4** (12 equiv, 12 μmol, 120 μL of a 0.1 M solution in MeOH), $CuSO_4$ (0.8 equiv, 0.8 μmol, 20 μL of a 40mM solution in H_2O), freshly prepared sodium ascorbate (4 equiv, 4 μmol, 80 μL of a 50mM solution in H_2O), and water (20 μL). The resulting preparation was treated in a sealed tube with microwave synthesizer Initiator from Biotage, set at 60°C and 100 W for 30 min with a 30 s premixing time to afford **5**. Temperature was monitored with an internal infrared probe. The solution was removed, and the CPG beads were washed with H_2O (2 mL) and MeOH (2 mL) and then dried.

15-mer oligonucleotide elongation, deprotection and purification: The beads were transferred into a column for DNA synthesizer. 5'-Cy3-Oligonucleotide 3'-tetrafucose **7** was synthesized using standard phosphoramidite chemistry with commercially available phosphoramidites (i.e. nucleosides and Cy3). The beads were placed into a sealed vial and treated with concentrated ammonia (1.5 mL) for 16 h at room temperature. The beads were filtered off and supernatant was withdrawn and evaporated to dryness. The residue was dissolved in water and purified by reverse phase HPLC on a C18 Delta Pak (15 μm) column (7.8× 300 mm; Waters) using a linear gradient of acetonitrile 16% to 36% in 0.05 M aqueous triethylammonium acetate (pH 7) for 20 min affording pure 5'-Cy3-3'-tetrafuco-oligonucleotide **7**: n=96 nmol (calculated by UV measurement at 260 nm, ε=152700); purity >98% (determined by HPLC at 254 nm); MALDI-TOF MS negative mode *m/z* calculated for $C_{245}H_{343}N_{69}O_{134}P_{17}$ $(M-H)^-$ 6924.36 found 6922.58.

2. Fabrication of DNA anchoring platform

2.1 Microreactors bearing glass slides.

Technology process of the device fabrication has been adapted from Mazurczyk *et al*[4]. Substrates were borosilicate glass slides (Schott GMBH). Photolithographic mask were laser printed plastic foils. Openings were squares (3 mm side). Spacing between the square were 4.5 mm in order to be compatible with the spotting robot and multi-canal micropipettes. Depth and surface roughness was measured using a mechanical profiler (Alfa-step 500 from KLA Tencor). The fabrication flow chart comprised the deposition of a chromium layer, a photolithographic step, opening of the chromium and glass etching. The following is a brief description of the fabrication process.

2.1.1 Photolithography

The substrates were cleaned successively with TDF4 detergent, a fresh Piranha mixture (sulphuric acid 95%: hydrogen peroxide 30%, 3:1 volume), rinsed with 18.2 M Ohm water and dried. A 150 nm chromium layer was deposited using magnetron sputtering (MRC system). SPR 220 4.5 photoresist (Shipley) was used for photolithography. Spinning parameters were 4000 rpm for 30s resulting in a 4 μm thick layer. Photolithography was carried out with an Karl Suss *MJB3* Mask Aligner.

2.1.2. Etching

Windows in the Cr layer were opened by wet etching (Merck chromium etchant). The oxide etching agents was a mixture of buffered oxide etchant (7/1, Hydro fluoridric acid, ammonium fluoride from Honeywell), hydrochloric acid and water (1/2/2, v/v). Etching was carried out at room temperature.

Finally, after the remaining chromium protective layers were removed with the use of chromium etchant (Merck) and acetone. The final device was a slide bearing 40 square microreactors (3 mm by side) with a depth of 60 μm (± 1μm).

The 4.5 mm spacing between each microwell was designed to be compatible with the subsequent use of microarrayer.

2.2 Silanisation of the glass slides.

Chemical functionalization (silanisation) is described in [5, 6]. Two types of substrates were used: flat borosilicate glass slide or microreactors bearing glass slides (3 mm squares).

In brief, after washing (fresh piranha, 20 min) the slides were heated under dry nitrogen for 2 h at 150 °C. Dry pentane was added at room temperature (250ml), followed by 300 µL of *tert*-butyl-11-(dimethylamino)silylundecanoate. After incubation at room temperature under dry nitrogen, pentane was evaporated and the slides were heated at 150 °C overnight and washed. The *tert*-butyl ester was converted into the corresponding acid by acidolysis (formic acid, 7h room temperature) followed by *N*-hydroxysuccinimide. (0.1 M, THF) and di(isopropyl)carbodiimide (0.1 M, THF) activation of the resulting carboxyl group. The slides were rinsed in THF and dichloromethane.

2.3 Immobilization of DNA strands

Amino-modified oligonucleotides were purchased from Eurogentec (Sequence 1 S1: 5'-GTGAGCCCAGAGGCAGGG-$(CH_2)_7$-NH_2 and Sequence 2 S2: 5' -GCTAATCCAACGCGGGCCAATCCTT - $(CH_2)_7$-NH_2).

- On flat slides, 0.3 µl of the desired sequence (25 µM in PBS 10 x, pH 8.5) was deposited in each microreactor.
- On 3 mm square microreactors bearing slides, amino modified nucleotides S1 and S2 (25 µM in PBS 10 x, pH 8.5) were deposited with a Biorobotics MicroGrid microarrayer (Digilab). The resulting microwell were featuring 32 spots of S1 and 32 spots of S2 (64 spots per well).

The covalent grafting between the amino-modified oligonucleotides was allowed to react with the carboxylic activated glass slides overnight at room temperature. Water was then allowed to slowly evaporate overnight at room temperature. The slides were washed with SDS 0.1% at 70°C for 30 min and rinsed with deionized water.

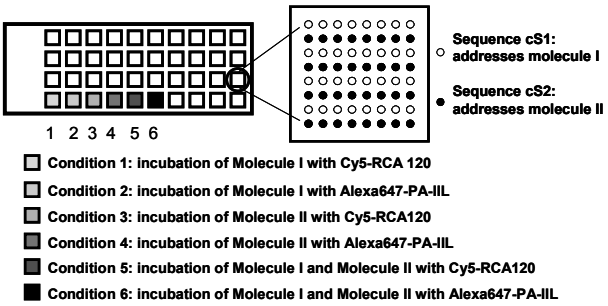


Figure S1: DNA sequence cS1 and cS2 are printed in alternative lines of eight spots at the bottom of each square well (40 wells per glass slide). cS1 and cS2 are complementary to the DNA tag S1 and S2 of **I** and **II** respectively. The glycomimetics are used either alone or together with lectins RCA120 or PA-IIL. The mixture is then incubated in one well.

3. Biological recognition

3.1 lectin labelling

3.1.1 Ricinus communis agglutinin 120 (RCA120) labelling with Cyanine 5 (Cy5)
The RCA120 lectin (Sigma) was labelled with Amersham Biosciences Cy5 Ab Labelling Kit according to the manufacturer's protocol. The labeled lectin was diluted in PBS 1× (pH 7.4), $CaCl_2$ (final concentration 5 μM) and 20% BSA (final concentration 2%) to the desired concentration. Protein concentration and the dye to protein ratio were estimated by optical density (nanodrop) reading the absorbance at 280 and 650 nm. Lectin concentration was estimated to be 4 μM bearing an average of 4 dyes per protein.

3.1.2 Labelling of PA-IIL lectin (gift from A. Imberty, CERMAV, Grenoble) with

Alexa 647

Labelling of PA-IL lectin with Alexa 647 was performed with the microscale labelling kit from Invitrogen. Protein concentration was estimated according to the manufacturer protocol by reading the absorbance at 280 and 650 nm. The final lectin concentration was estimated at 24.5 µM with a degree of labelling of 0.04.

3.2 In solution" biological recognition

Glycoconjugates (1 µM final concentration) were incubated with desired concentration of labelled lectins (0.3 µM µM for RCA120 and 0.4 µM µM for PA-IIL) in PBS-Tween20 0.02% solution. BSA (2% final concentration) and $CaCl_2$ (1µg/ml final concentration) were added into the solution respectively.

- On flat substrates, 40 µL of each solution was placed in each well of the slide which covered with CoverWell. The samples were allowed to react for 2h at 37 °C in a water saturated chamber. The slides were then washed with PBS1×(pH 7.4)-Tween20 0.02% for 5min and scanned.
- On microreactor bearing microwell, 2 µl of a solution comprising the glycoconjugate(s) (1 µM final concentration), BSA (2% final concentration, $CaCl_2$ (1µg/ml final concentration) and labelled lectin (0.3 µM final concentration for RCA120 and 0.4 µM for PA-IIL) was deposited at the bottom of each well. The samples were allowed to react for 2h at 37 °C in a water saturated chamber. The slides were then washed with PBS1×(pH 7.4)-Tween20 0.02% for 5min and scanned.

3.3 Fluorescence scanning

Slides were scanned with the Microarray scanner, GenePix 4100A software package (Axon Instruments) at wavelengths of 532 and 635 nm. The fluorescence signal of each conjugate was determined as the average of the mean fluorescence signal of three spots

Comment about Figure 4

In Figure 4a, error bars are not present for condition 2 and 4 for Sequence cS1 and Sequence cS2 due to a saturation of the signal. Therefore, the error bars are equal to zero.
For condition 3, sequence cS1, the error bar are too small to be visualised -/+94 a. u..
In Figure 4b, error bars are below -/+12 a. u..

References

1. Y. Chevolot, C. Bouillon, S. Vidal, F. Morvan, A. Meyer, J. P. Cloarec, A. Jochum, J. P. Praly, J. J. Vasseur and E. Souteyrand, *Ang. Chem. Int. Ed.*, 2007, **46**, 2398-2402.
2. J. Lietard, A. Meyer, J. J. Vasseur and F. Morvan, *J. Org. Chem.*, 2008, **73**, 191-200.
3. F. Morvan, A. Meyer, A. Jochum, C. Sabin, Y. Chevolot, A. Imberty, J.-P. Praly, J.-J. Vasseur, E. Souteyrand and S. Vidal, *Bioconjugate Chem.*, 2007, **18**, 1637-1643.
4. R. Mazurczyk, G. El Khoury, V. Dugas, B. Hannes, E. Laurenceau, M. Cabrera, S. Krawczyk, E. Souteyrand, J.-P. Cloarec and Y. Chevolot, *Sensor. Actuator. B*, 2008, **128**, 552-559.
5. Y. Chevolot, E. Laurenceau and E. Souteyrand, *ITBM-RBM*, 2007, **28**, 194-209.
6. V. Dugas, G. Depret, B. Chevalier, X. Nesme and E. Souteyrand, *Sensor. Actuator. B*, 2004, **101**, 112-121.