Supplementary Material for Chemical Communications

Microarrays of Heparin Oligosaccharides Obtained by Nitrous Acid Depolymerization of Isolated Heparin

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General Information. All chemicals used were reagent grade and used as supplied. All aqueous solutions were made from nanopure water. Solutions used for chip hybridizations were sterile filtered through a 0.2 μm syringe filter prior to use. Amine-coated glass slides were Corning GAPS II slides and were purchased from Corning Inc. Deaminated heparin (5 KDa) was purchased from Sigma-Aldrich. Human AT-III (58 KDa) was purchased from Haematologic Technologies Inc. BSA was purchased from Fluka. MICROCON centrifugal filter units were purchased from Millipore. Basic Fibroblast Growth Factor (FGF-2) and Acidic Fibroblast Growth Factor (FGF-1) were purchased from PeproTech EC. Microarrays were constructed using a Perkin Elmer noncontact printer. For all incubations, 90 μL of protein solution was applied to the slide by using HybriSlip Hybridization Covers from Grace BioLabs. Slides were scanned using a LS400 scanner from Tecan and quantified using Scan Array Express Software. All data are the average signal of at least five spots on a single array; errors are the standard deviations of those measurements.

Heparin Labeling.

Deaminated heparin (1 mg, 5 KDa) was dissolved in 100 mM sodium bicarbonate buffer (pH 9.0, 1mL). A solution of FITC in a 9:1 bicarbonate buffer/DMF mixture (250 μL, 1 mg/mL) was added to the heparin solution and the reaction mixture was incubated in the dark for 1 h. To remove the uncoupled fluorescent probe, the solution was submitted to centrifugal ultrafiltration (MICROCON 3 KDa, 40 min, 14000 rpm), washing three times with bicarbonate buffer. The residue was diluted with 100 mM bicarbonate buffer (500 μL) and stored at -20ºC until use.

Heparin Array Fabrication: Manual Spotting.

Deaminated heparin oligosaccharides as well as FITC-labelled heparin were dissolved in 100 mM sodium bicarbonate buffer (pH 9.0) and manually printed on amine-coated GAPS slides by using a micropipette (0.25-0.5 μL per spot). The slides were incubated overnight in a small humid chamber at room temperature and then dried for 1 h and rinsed three times with distilled water (25 mL). To quench all remaining amine groups, the slides were immersed in a 3:1 bicarbonate buffer/DMF mixture (40 mL) containing carboxybenzaldehyde (38 mg). The slides were incubated in this solution for 2 h, washed three times with distilled water (25 mL) and then centrifuged at 1700 rpm for 5 min to ensure complete dryness. When FITC heparin was used, the stability of the imine bond was tested by scanning the retained fluorescence signal after incubation in PBS buffer containing BSA (1%, w/v) for 2 h (Figure 1).

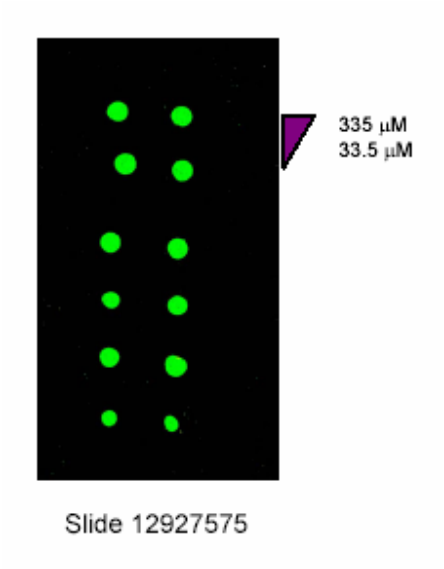

Figure 1. Fluorescence images of FITC heparin microspots after incubation in PBS buffer and extensive washing.

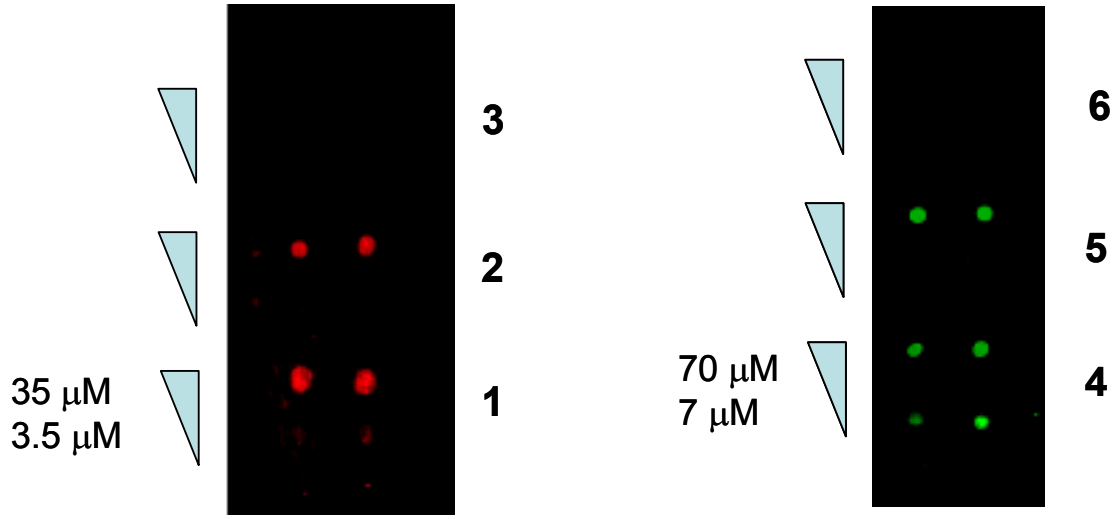

Figure 2. Fluorescence images of heparin arrays containing oligosaccharides **1-6** (manual spotting) after incubation with fluorescent-labelled AT-III.

Heparin Array Fabrication: Robotic Printing

After optimizing the immobilization chemistry, miniaturization of the spotting process was performed by using an arraying robot:

- FITC-labelled deaminated heparin was dissolved in sodium bicarbonate buffer (100 mM, pH 9.0) and spotted at an initial concentration of 16 mg/mL and then diluted in serial dilutions of 1:4. Data of ten repeats of the same experiment were statistically analyzed.
- Heparin octasaccharides **1-3** and decasaccharides **4-6** were used in three different concentrations ranging from 35 μM to 0.35 μM. All samples were printed in five replicates.

The robot delivered 1 nL of carbohydrate-containing solutions to create spots with a diameter of ~200 μm. After printing, slides were incubated overnight, quenched and washed as described above.

Immobilization of FITC Heparin Using Reductive Amination Conditions

FITC heparin was dissolved in MES buffer (50 mM, pH 6.8) at concentrations ranging from 16 mg/mL to 0.24 μg/mL. Immediately before printing, an aqueous $NaCNBH_3$ solution (2 mg/mL) was added (1:4 v/v) to the dilution series. The heparin solutions were spatially arrayed onto GAPS slides and incubated overnight. After washing with water, unreacted amines were quenched with a 3:1 bicarbonate buffer/DMF mixture (40 mL) containing carboxybenzaldehyde (38 mg). The slides were incubated in this solution for 2 h, washed three times with water and centrifuged to ensure complete dryness.

Protein Labeling[1]

AT-III Labeling with FITC.

The protein (250 μg) was dissolved in 100 mM sodium bicarbonate buffer (pH 9.0, 250 μL). An excess of a diluted solution of FITC in a 9:1 bicarbonate buffer/DMF mixture (50 μL, 1 mg/mL) was added to

the AT-III solution and the reaction mixture was incubated in the dark with slow stirring for 1 h. To remove the uncoupled fluorescent probe, the solution was submitted to centrifugal ultrafiltration (MICROCON 30 KDa, 40 min, 14000 rpm), washing three times with bicarbonate buffer. The residue was finally diluted with a 1:1 mixture of water and glycerol (500 μL) and stored at -20ºC until use.

FGF Labeling with Fluorescein Maleimide.

The protein (50 μg) was dissolved in 10 mM PBS buffer (pH 7.4, 250 μL). An excess of a diluted solution of fluorescein maleimide in a 9:1 PBS buffer/DMF mixture (31 μL, 1 mg/mL) was added to the FGF solution and the reaction mixture was incubated in the dark for 2 h. To remove the excess of fluorescent probe, the solution was submitted to centrifugal ultrafiltration (MICROCON 10 KDa, 30 min, 14000 rpm), washing three times with 10 mM PBS. The residue was finally dissolved in a mixture of PBS buffer (250 μL) and glycerol (50 μL) and stored at -20ºC until use.

Array Incubation and Analysis

An aliquot of the labeled protein solution (10 μL, 2-5 μg) was diluted with PBS (10 mM, 90 μL) containing BSA (1%, w/v). The protein solution was placed over the slide, using a glass cover slip to allow the solution to be distributed evenly. Arrays were incubated at room temperature for 1 h. To remove unbound protein, chips were placed in PBS (10 mM) containing BSA (0.1%, w/v) and Tween 20 (1%, w/v) for at least 5 min, and then immersed in water (50 mL) three times and centrifuged to dryness. Slides were scanned by using a LS400 scanner and quantified using Scan Array Express Software. All data are the average signal of at least five spots on a single array; errors are the standard deviations of those measurements.

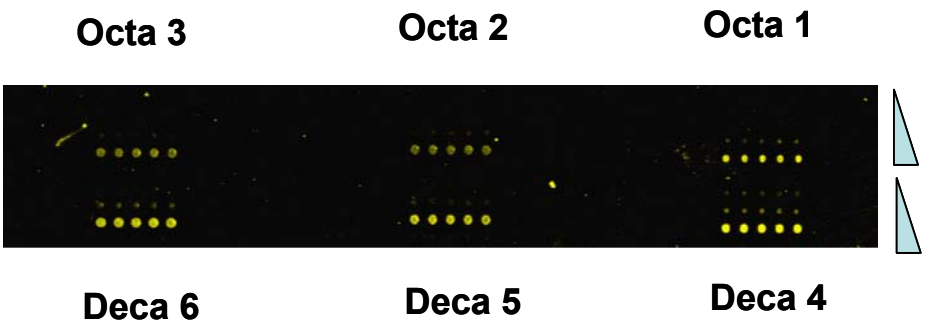

Figure 3. Microarray after incubation with FGF-1.

Notes and References.

1. Alternatively, a sandwich procedure in which the bound protein is detected with labelled secondary antibodies can also be used, as described in J. L. De Paz, C. Noti and P. H. Seeberger, *J. Am. Chem. Soc.,* 2006, **128**, 2766-2767.