Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2008

Supporting Information

Surface patterning of (bio)molecules onto the inner wall of fused-silica capillary tubes

Nabil Dendane,[a, b] Antoine Hoang,[b] Olivier Renaudet,[a] Françoise Vinet,[b] Pascal Dumy,[a] and Eric Defrancq,[a]*

[a] Département de Chimie Moléculaire - UMR CNRS 5250, ICMG FR2607, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 9, (France)

[b] LETI-CEA, 17 rue des Martyrs, 38054 Grenoble Cedex 9, (France)

Experimental Section

Materials and methods. All solvents and reagents used were of highest purity available. *O*-[2-(6-oxocaproylamino)ethyl]-*O'*-methylpolyethylene glycol (MW = 2000), FITC labelled anti-HA antibody, anti-SDF-1, Concanavalin A and Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich. The complementary Cy3 labelled oligonucleotide was purchased from MWG Biotech and the hybridization solution from Sigma. Flexible fused-silica capillaries with UV-transparent coating (interior diameter: 100 μm) were obtained from Polymicro Technologies (Phoenix, AZ). The irradiation experiments were performed on an Olympus inverted microscope model IX60, equipped with a 100W mercury lamp and a 365 nm interference filter. The patterning was performed by irradiation of the focused zone. Scanning experiments were carried out using a Gentac Scanner (Genomic Solution®). RP-HPLC analysis and purification of peptides and glyco-peptide were performed on a Waters HPLC system with dual wavelength detector using the following solvent system: solvent A, water containing 1% TFA; solvent B, acetonitrile containing 1% TFA and 10% H_2O with a linear gradient (5 to 100% B in 15 min.). UV absorbance was monitored at 214 nm and 250 nm simultaneously.

Synthesis.

Oligonucleotide Seq1. The oligonucleotide sequence employed in the present work is 5'-d-XTTTTTGATAAACCCACTCTA-3' (**Seq1**) where X represents the 5'-aldehyde linker. It was prepared as previously reported.[1]

Glyco-peptide 2. The glyco-peptide **2** was prepared by adapting the previously reported method.[2] A brief protocol is reported below.

To a solution of cyclodecapeptide bearing aldehyde functions (2 mg, 1.6 mmol) in 10% aqueous acetic acid (1 mL), aminooxy α-D-mannopyranosyl (3 mg, 16 mmol) was added. After stirring 1 h at room temperature, the reaction mixture was lyophilized. The crude tetravalent glyco-peptide was then dissolved in DMF (1 mL) and levulinic pentafluorophenol ester (1 mg, 3.2 mmol) was added. The pH of the solution was adjusted at 8 with DIEA. The reaction mixture was stirred for 30 minutes at room temperature. Purification by RP-HPLC afforded the glyco-peptide **2** as a white powder in a 52% yield (1.7 mg). ES-MS (positive mode): calcd for $C_{84}H_{135}N_{19}O_{40}K$ 2088.9 $[M+H]^+$, found: 2088.6.

Peptide 5: Ser-HA peptide H-SGYPYDVPDYAGYPYDVPDYAGYPYDVPDYAS-NH_2 **4** was prepared in an automated peptide synthesizer (ABI433A, Applied BioSystem) by using the standard Fmoc/*t*Bu chemistry. Synthesis was performed on a 0.1 mmol scale on a MBHA resin. The peptide was cleaved from the resin and deprotected upon acidic treatment (TFA/water/TIS/EDT/*p*-cresol: 90/2.5/2.5/2.5/2.5) for 2 h at room temperature. The peptide was purified by RP-HPLC on a 7 μm C18 Nucleosil column by using a linear water/acetonitrile gradient containing TFA (0.09% *v/v*; 22 $mLmin^{-1}$, detection at 214 nm). ES-MS (positive mode): calcd for $C_{171}H_{216}N_{32}O_{56}$ 3613.5 $[M+H]^+$, found: 3614.9. Subsequent oxidation of the *N*-terminal serine residue was performed using $NaIO_4$ (1.5 equiv) in water for 1 h. The glyoxylic peptide **5** was used without further purification for surfaces immobilization. ES-MS (positive mode): calcd for $C_{170}H_{212}N_{32}O_{55}$ 3581.5 $[M+H]^+$, found: 3581.6.

General protocol for aminooxy patterning of glass surfaces inside the capillary tubes.

Step 1 - Hydratation of the capillary tube: The capillary tube was filled with an aq. ethanolic solution of NaOH (1g NaOH, 4 mL H_2O, 3 mL EtOH) for 1 h, and washed successively with ultra-pure H_2O, 0.2 N aq. HCl and H_2O. The capillary tube was then dried under nitrogen.

Step 2 - Silanization: The capillary tube was filled with a 10 mM solution of silane **1** in trichloroethylene and incubated for overnight at room temperature. The capillary was then washed

with trichloroethylene followed by EtOH and dried under nitrogen. Finally, the capillary tube was cured at 110 °C for 3 h.

Step 3 - Photodeprotection: The capillary tube was filled with a 5% aq. pyridine solution and was mounted on the microscope stage. The irradiations were performed using a mercury lamp (100 W, 24 $mW.cm^{-2}$) and were focused at different positions on the capillary. Each irradiation thus corresponded to a separate spot on the capillary array. The capillary was then washed with H_2O and dried under nitrogen.

Immobilization protocol.

Carbohydrates **2** *and* **3**. The capillary was filled with a 10% acetic acid solution of glyco-peptide **2** (400 μM) for 1 hour or with a 0.4 M ammonium acetate buffer solution of GAG **3** (20 μM) for 2 min at room temperature. A constant reagent flow (10 $\mu L.min^{-1}$) was applied by using a syringe pump. The capillary was then successively washed with H_2O, 1% aq. SDS solution, H_2O and finally dried under nitrogen. Bovin serum albumin solution (6 $mg.mL^{-1}$) was next passed through the tube for 45 min and the capillary tube was then washed with 10 mM phosphate buffer (pH 7.4).

HA Peptide **5**. The capillary was filled with an acetonitrile/0.4 M ammonium acetate buffer solution (1/1:*v/v*) of HA peptide **5** (20 μM) for 2 min at room temperature. A constant reagent flow (10 $\mu L.min^{-1}$) was applied by using a syringe pump. The capillary was then successively washed with H_2O, 1% aq. SDS solution, H_2O and finally dried under nitrogen. Bovin serum albumin solution (6 $mg.mL^{-1}$) was next passed through the tube for 45 min and the capillary tube was then washed with 10 mM phosphate buffer (pH 7.4).

Tridecanal and pentafluorobenzaldehyde. The capillary was filled with a toluene solution of tridecanal or pentafluorobenzaldehyde (100 mM) for 1 hour at room temperature. A constant reagent flow (10 μL.min-1) was applied by using a syringe pump. The capillary was then successively washed with H_2O, 1% aq. SDS solution, H_2O and finally dried under nitrogen. The capillary tube was then fully irradiated by using a mercury lamp (100 W, 24 $mW.cm^{-2}$) for 10 sec. The capillary was next filled with a 0.4 M ammonium acetate buffer solution containing oligonucleotide-5'-aldehyde (**Seq1**, 20 μM) for 2 min at room temperature. A constant reagent flow (10 $\mu L.min^{-1}$) was applied by using a syringe pump. The capillary was successively washed with H_2O, 1% aq. SDS solution, H_2O and finally dried under nitrogen.

Polyethylene glycol: The capillary was filled with a 0.4M ammonium acetate buffer of PEG 5 (20 μM) for 2 min at room temperature. A constant reagent flow (10 $\mu L.min^{-1}$) was applied by using a

syringe pump. The capillary was then successively washed with H_2O, 1% aq. SDS solution, H_2O and finally dried under nitrogen. The capillary tube was then fully irradiated by using a mercury lamp (100 W, 24 mW.cm^{-2}) for 10 sec. The capillary was next filled with a 0.4 M ammonium acetate buffer solution containing oligonucleotide-5'-aldehyde (**Seq1**, 20 µM) for 2 min. A constant reagent flow (0.1-10 µL.min-1) was applied by using a syringe pump. The capillary was successively washed with H_2O, 1% aq. SDS solution, H_2O and finally dried under nitrogen.

Binding studies.

For mannose residues: The capillary was filled with a solution of FITC labelled concanavalin A (1 mg.mL^{-1}) in 10 mM phosphate buffer (pH 7.4) for 1 hour at 39°C. The capillary was then washed with 10 mM phosphate buffer and scanned.

For heparine residues: The capillary was filled with a solution of FITC labelled SDF-1 chimiokine (20 nM) in 10 mM phosphate buffer (pH 7.4) for 1 hour at 39°C. The capillary was then washed with 10 mM phosphate buffer and scanned.

For peptide HA: The capillary was filled with a solution of FITC labelled HA antibody (10 µg.mL^{-1}) in 10 mM phosphate buffer (pH 7.4) for 1 hour at 39°C. The capillary was then washed with 10 mM phosphate buffer and scanned.

For oligonucleotide (hybridization): The capillary was filled with the hybridization solution of Cy3® labelled complementary oligonucleotide (10 nM) for 1 hour at 39 °C. The capillary was then washed with 2X SSC buffer and scanned.

References

[1] O. P. Edupuganti, Y. Singh, E. Defrancq, P. Dumy, *Chem. Eur. J.* **2004**, *10*, 5988.

[2] a) O. Renaudet, P. Dumy, *Org. Lett.* **2003**, *5*, 243; b) Y. Singh, O. Renaudet, E. Defrancq, P. Dumy, *Org. Lett.* **2005**, *7*, 1359; c) M. Wilczewski, A. Van der Heyden, O. Renaudet, P. Dumy, L. Coche-Guérente, P. Labbé, *Org. Bioorg. Chem.* **2008**, *6*, 1114.