

3D Small-Molecule Microarrays Supplementary Information

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Chapter 1

Supplementary Information

1.1 General Information

Experimental techniques and apparatus as standard except as otherwise indicated. Reactions were carried out under nitrogen with dry, freshly distilled solvents. Dichloromethane was distilled from calcium hydride. Diethyl ether and THF were freshly distilled from sodium benzophenone ketyl under argon. All other reagents were purified in accordance with the instructions in "*Purification of Laboratory Chemical*¹" or used as obtained from commercial sources (Aldrich, unless stated otherwise).

Yields refer to chromatographically and spectroscopically pure compounds. All reactions were monitored by thin layer chromatography (TLC) using glass plates precoated with Merck silica gel 60 F254 or aluminum oxide 60 F254. Visualisation was carried out using UV fluorescence ($\lambda = 254$ nm) or by staining with ammonium molybdate or potassium permanganate. Retention factors (R_f) are quoted to two decimal places.

Melting points were obtained using a Reichert hot plate microscope with a digital thermometer attachment and are uncorrected.

Infrared spectra were recorded neat on a Perkin-Elmer Spectrum One spectrometer with internal referencing. Absorption maxima (max) are reported in wavenumbers (cm^{-1}) and the following abbreviations are used: w, weak; md, medium; st, strong; br, broad.

Proton magnetic resonance spectra were recorded on Bruker Ultrashield 400 or 500 MHz spectrometers. Proton assignments are supported by 1H-1H spectra where necessary. Chemical

shifts ($^1\text{H-NMR}$: δ) are quoted in ppm and are referenced to the residual non-deuterated solvent peak. Chemical shifts were measured relative to chloroform (^1H d 7.26, ^{13}C d 77.36), methanol (^1H d 3.34, ^{13}C d 49.86) or DMSO (^1H d 2.54, ^{13}C d 40.45) as appropriate. Coupling constants (J) are reported to the nearest 0.5 Hz. Data are reported as follows: chemical shift, integration, multiplicity [br, broad; s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; sext, sextuplet; sept, septuplet; m, multiplet; or as a combination of these (e.g. dd, dt, etc.)], coupling constant(s) and assignment.

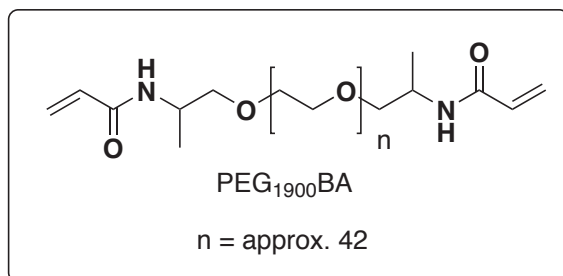
Carbon magnetic resonance spectra were recorded on Bruker Ultrashield 400 or 500 MHz spectrometers. Carbon spectra assignments are supported by DEPT editing. Chemical shifts ($^{13}\text{C-NMR}$: δ) are quoted in ppm to the nearest 0.1 ppm, and are referenced to the deuterated solvent.

LCMS spectra were recorded on an HP/Agilent LCMS APCI 120-1000 full gradient machine. High resolution mass measurements were made by the EPSRC mass spectrometry service (Swansea) on a Finnigan MAT 95XP spectrometer or the departmental Waters LCT machine.

Bis-NHS-Cy3, repel silane (dimethyl-dichloro-silane (2% w/v) in octamethylcyclotetrasiloxane) and Codelink slides were purchased from GE Healthcare. Microscope slides (S8400) and cover wells (PC200) were purchased from Sigma-Aldrich. A Varian Cary UV-Vis Spectrometer Bio 100 was used to obtain UV-Vis spectra. A Veeco atomic force microscope (AFM) was used with Nanoscope V5 AFM software. AFM tips (Olympus cantilever OMCL-AC160TS) were obtained from Atomic Force GmbH.

1.2 Synthesis of PEG Cross-Linker

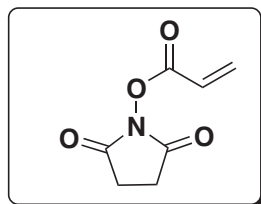
1.2.1 Bis-2-acrylamidoprop-1-yl-PEG₁₉₀₀ (PEG₁₉₀₀BA) 1



Bis-2-aminoprop-1-yl-polyethyleneglycol₁₉₀₀ (30.0 g, 1 eq., 15.8 mmol) was dissolved in CH₂Cl₂ (60 mL) and triethylamine (4.4 mL, 2 eq., 31.6 mmol) at 0 °C. Acryloyl-chloride (2.57 mL, 2 eq., 31.6 mmol) was added drop wise and the reaction was stirred at 0 °C for one hour. The reaction mixture was filtered to remove the triethylamine-hydrochloride salt. The filtrate was washed with water (2 x 20 mL) and the organic layer concentrated to half volume under reduced pressure. Diethyl ether (20 mL) was added and the reaction mixture concentrated under reduced pressure to precipitate out the product. The precipitate was filtered and washed with diethyl ether, before drying to give the product **1** as a white powder³ (38.4 g, 81%): $\nu_{max}(\text{neat})/\text{cm}^{-1}$ 2882br, 1667w, 1466m, 1104st; δ_H (400 MHz, CDCl₃) 6.28-6.23 (2H, m, CH=CH'H'' x 2), 6.23-6.15 (2H, m, CH₂=CH x 2), 5.58-5.56 (2H, m CH=CH'H'' x 2), 4.20-4.16 (2H, m, CHN x 2), 3.65-3.59 (168H, m, -OCH₂CH₂O-), 3.50 (4H, m, CH₂ x 2), 1.19-1.16 (6H, m, CH₃ x 2). Literature data in agreement.³

1.3 Synthesis of NHS Ester Monomer Unit

1.3.1 Acryloyloxy-succinimide **2**



N'-Hydroxysuccinimide (25 g, 1 eq., 217 mmol) was dissolved in chloroform (175 mL) and triethylamine (33.3 mL, 1.1 eq., 238.7 mmol). Acryloyl-chloride (17.65mL, 1 eq., 217 mmol) was added slowly dropwise at 0 °C. The reaction mixture was stirred for 3 hours. The reaction mixture filtered and washed with water (2 x 150 mL) and the organic layer dried with MgSO₄. The reaction mixture was concentrated under reduced pressure to give a white powder which was recrystallised from a solution of EtOAc:Hexane (1:1) to give a white crystalline product **2** (25.86 g, 74%): mp. 68-69 °C (Literature² 69 °C); *R_f* 0.31 (SiO₂, 1:1 EtOAc:Hexane); *v_{max}*(neat)/cm⁻¹ 1731br, 1209m, 1092st, 995st; δ_H (400 MHz, CDCl₃) 6.66 (1H, dd, *J* 17.5, 1.0 Hz, COCH=CH), 6.40 (1H, dd, *J* 17.5, 11.0 Hz, COCH=CH₂), 6.23-6.20 (1H, dd, *J* 11.0, 1.0 Hz, COCH=CH'), 2.85-2.80 (4H, m, 2 x CH₂); δ_C (125 MHz, CDCl₃) 169.2, 161.1, 136.2, 122.9, 25.6; *m/z* HRMS found 194.0422, C₇H₇NO₄Na (MNa⁺), requires 194.0429. Literature data in agreement.²

1.4 Manufacture of 3D hydrogel slides

1.4.1 Preparation of bind-silane glass

To clean slides, 100 mL of piranha solution was made by addition of 70 mL of c.H₂SO₄ in aliquots to 30 mL of 35% v/v H₂O₂ in a 250 mL Buchner flask which itself was held in an ice bath. Standard microscope glass slides were placed in a crystallisation dish and covered carefully with 100 mL of piranha solution. The crystallisation dish was covered with a larger crystallisation dish and left overnight. The piranha solution was diluted into a large excess of water, and the slides were washed with distilled water (4 x 200 mL) by gentle shaking and finally rinsed with 99% ethanol. Cleaned slides were used straight away. **Caution:** *Piranha solution reacts violently with organic compounds like acetone and should be handled with extreme care. Wear thick gloves, lab coat and safety glasses and handle exclusively in a fume hood at all times.*

Cleaned glass slides were treated with a "bind silane" solution (0.3% v/v methylacryloxypropyltrimethoxysilane) (GE Healthcare Ltd.) to give a methacryloyl functionality. For a 10 mL solution this consisted of ethanol (8.27 mL) glacial acetic acid (200 μ L), bind silane (30 μ L) and distilled water (1.5 mL). The bind silane solution was pipetted onto cleaned glass slides, and covered for 90 minutes. The slides were then rinsed with 99% ethanol and allowed to air dry in a sealed container.

1.4.2 Preparation of Hydrophobic Glass

Repel silane (dimethyl-dichloro-silane (2% w/v) in octamethylcyclotetrasiloxane) (GE Healthcare Ltd.) was pipetted onto ordinary glass slides and left for 5-10 minutes to give water repellent properties to the glass. Repel silane slides were rinsed with 99 % ethanol and air dried.

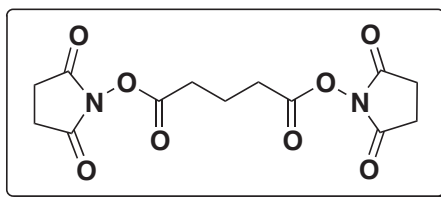
1.4.3 Incorporation of Gels Onto Functionalised Glass

- 1) The polymerisation precursor solution was made consisting of 90 mg PEG₁₉₀₀BA **1**, 90 mg acryloyloxysuccinimide **2**, 90 μ L acryloylmorpholine **3**, 30 μ L hydroxy-2-methylpropiophenone **4** and 2.7 mL dimethylformamide (DMF). The mixture was sonicated for 5 minutes before degassing for 15 minutes using nitrogen.
- 2) 150 μ L of the degassed polymer mixture was pipetted along the long edge of a functionalised

bind silane slide. The repel silane slide was inverted and carefully placed sandwiching the bind silane slide at an angle to create a flap at the apex between the two slides. The repel silane slide was carefully lowered using tweezers to sandwich the bind slide so the mixture spread evenly over the slide.

- 3) The slides were transferred to a UV box (Stratagene Stratalinker) on a platform 6 cm from the light source (254nm) and irradiated for 20 minutes.
- 4) Following polymerisation, the repel silane top glass slide was removed with care using a scapel and the gel slide was rinsed with 99% ethanol. Gel slides were then placed in a crystallisation dish facing gel side up and washed in ethanol for 10 mins using an Orbital Shaker S01 (Stuart Scientific) at a speed of 150 rpm. Slides were removed and dried under a stream of nitrogen and stored in a vacuum desiccator for up to one month before use.

1.5 Synthesis of Disuccinimidylglutarate (DSG)



Hydroxysuccinimide (1.36 g, 2 eq., 11.8 mmol) and triethylamine (1.64 mL, 2 eq., 11.8 mmol) were dissolved in CH_2Cl_2 (30 mL). Glutaric acid chloride (1.0 mL, 1 eq., 5.9 mmol) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 12 hours. The reaction was filtered and the solvent was removed under reduced pressure. The crude material was recrystallised by dissolving it in the minimum amount of hot CH_2Cl_2 before precipitating it dropwise with hexane. The product was filtered and dried to give the final product DSG as an off white crystalline powder⁴ (1.68 g, 89% yield): mp. 144-146 °C (no lit data⁴); R_f 0.35 (SiO_2 , 1:1 EtOAc: CH_2Cl_2); ν_{max} (neat)/ cm^{-1} 1813w, 1783w, 1732st, 1359w, 1202st, 1087m, 1069m, 1048m, 866m; δ_H (400 MHz, CDCl_3) 2.83 (8H, br s, 2 × $\text{OCCH}_2\text{CH}_2\text{CO}$), 2.79 (4H, t, J 7.5 Hz, $\text{CH}_2\text{CH}_2\text{CH}_2$) 2.19 (2H, quin, J 7.5 Hz, $\text{CH}_2\text{CH}_2\text{CH}_2$); δ_C (100 MHz, CDCl_3) 169.3 & 168.1 (2 × $\text{C}=\text{O}$), 30.0, 26.0 and 20.0 (3 × CH_2); m/z (ES^+) 327.1 (MH^+).

1.6 Manufacture of 2D NHS ester slides

1.6.1 Preparation of Amino Functionalised Glass

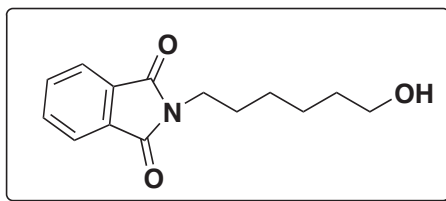
Ordinary glass slides were cleaned using piranha solution outlined previously. Cleaned slides were immersed in a staining jar containing a solution of 5% aminopropyltrimethoxysilane in absolute ethanol (200 mL). The solution was stirred for 10 minutes at room temperature. The slides were removed and rinsed with 95% ethanol and left to dry in the air for a further 10 minutes. The slides were then placed in a vacuum oven for two hours at 80 °C. The amino modified slides were removed and allowed to cool to room temperature. Amino modified slides were stored in a vacuum desiccator for up to month before use.⁴

1.6.2 Preparation of 2D NHS Slides

Amino modified slides were immersed in a different staining jar containing a solution of disuccinimidylglutarate (DSG) (653 mg, 2 mmol) dissolved in CH₂Cl₂ (180 mL) and diisopropylethylamine (20 mL, 1% v/v). The solution was stirred for two hours at room temperature. The NHS ester slides were removed and washed three times with CH₂Cl₂ and dried under a stream of nitrogen. NHS ester slides were stored in a vacuum desiccator for up to one month before use.⁴

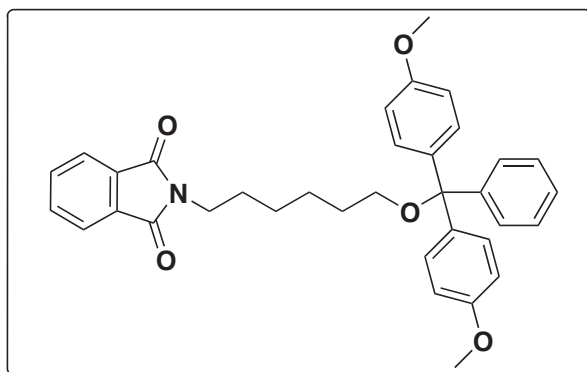
1.7 Synthesis of Dimethoxytrityl-6-amino-1-hexanol 5

1.7.1 6-N-Phthaloyl-6-amino-1-hexanol 5A



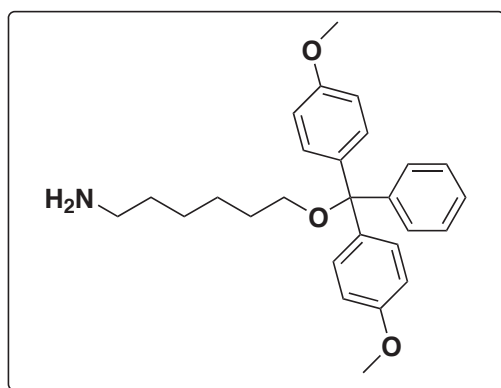
To a solution of 6-chloro-1-hexanol (1.0 g, 1 eq., 7.3 mmol) in DMF (50 mL) was added potassium phthalimide (1.5 g, 1.1 eq., 8.1 mmol). The reaction mixture was heated at 60 °C for four hours and allowed to cool to room temperature before filtering. The solvent was removed under reduced pressure and the residue redissolved in CH₂Cl₂ (10 mL). The organic solution was washed with 5% aqueous NaHCO₃ (2 × 5 mL) and saturated NaCl (5 mL). The organic layer was dried over MgSO₄ and concentrated under reduced pressure to yield the product **5A** without further purification as a pale yellow oil (752 mg, 50%): *R_f* 0.37 (SiO₂, CH₂Cl₂); *v_{max}*(neat)/cm⁻¹ 3362br, 2926w, 1769w, 1709st, 1396m, 1043m, 723st; *δ_H* (400 MHz, CDCl₃) 7.90-7.85 (2H, m, Ar-H), 7.75-7.71 (2H, m, Ar-H), 3.72 (2H, t, *J* 7.0 Hz, CH₂N), 3.66 (2H, t, *J* 6.5 Hz CH₂OH), 1.76-1.68 (2H, quin, *J* 7.5 & 7.0 Hz, CH₂CH₂OH) 1.63-1.57 (2H, quin, *J* 7.0 & 6.5 Hz, NCH₂CH₂), 1.50-1.36 (4H, m NCH₂CH₂CH₂CH₂); *δ_C* (100 MHz, CDCl₃) 168.5 (C=O), 132.2 (quaternary), 133.8 & 123.2 (2 × CH), 62.7, 37.8, 32.5, 28.5, 26.5 & 25.2 (6 × CH₂); *m/z* (ES⁺) 248.2 (MH⁺). Literature data in agreement.⁵

1.7.2 1-O-4,4'-Dimethoxytrityl-6-N-phthaloyl-6-amino-1-hexanol **5B**



Triethylamine (1.05 mL, 2.5 eq., 7.57 mmol) was added to a stirred solution of 6-*N*-phthaloyl-6-amino-1-hexanol **5A** (752 mg, 1.0 eq., 3.03 mmol), 4,4'-dimethoxytrityl-chloride (1.12 g, 1.1 eq., 3.3 mmol) and DMAP (37 mg, 0.1 eq., 0.30 mmol) in CH₂Cl₂ (20 mL). The reaction mixture was stirred for 12 hours at room temperature and quenched with 5% aqueous NaHCO₃ and stirred for a further 30 minutes. The organic layer was extracted with CH₂Cl₂ (2 x 10 mL), washed with saturated NaCl, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified using flash chromatography (CH₂Cl₂) to give the product **5B** as a thick orange oil (644 mg, 39% yield): *R_f* 0.29 (SiO₂, CH₂Cl₂); *v_{max}*(neat)/cm⁻¹ 2935br, 1772w, 1708st, 1607w, 1508m, 1246m, 1032st, 827m, 719st; *δ_H* (400 MHz, CDCl₃) 7.76-7.74 (2H, m, Ar-H), 7.62-7.60 (2H, m, Ar-H), 7.34 (2H, d, *J* 7.0 Hz, Ar-H), 7.24-7.16 (6H, m, Ar-H), 7.10 (1H, t, *J* 7.5 Hz, Ar-H), 6.73 (4H, d, *J* 9.0 Hz, Ar-H), 3.70 (6H, s, OCH₃ x 2), 3.58 (2H, t, *J* 7.5 Hz CH₂N), 2.95 (2H, t, *J* 6.5 Hz, CH₂O), 1.63-1.48 (4H, m, NCH₂CH₂CH₂CH₂CH₂CH₂O), 1.20-1.36 (4H, m, NCH₂CH₂CH₂CH₂CH₂CH₂O); *δ_C* (100 MHz, CDCl₃) 168.8 (C=O), 158.7, 145.8, 137.1, 132.6 & 86.1 (5 x quarternary), 134.2, 130.4, 128.6, 128.1, 126.9, 123.6 & 113.4 (7 x CH), 63.7, 38.4, 30.4, 29.0, 27.2 & 26.3 (6 x CH₂), 55.6 (1 x CH₃); *m/z* (ES⁺) 247.3 (MH⁺-trityl). Literature data in agreement.⁵

1.7.3 1-O-4,4'-Dimethoxytrityl-6-amino-1-hexanol **5**



To a stirred solution of 1-*O*-4,4'-dimethoxytrityl-6-*N*-phthaloyl-6-amino-1-hexanol **5B** (630mg, 1 eq., 1.1 mmol) in MeOH (10 mL) was added hydrazine hydrate (60 μ L, 1.1 eq., 1.2 mmol). The reaction mixture was stirred for 16 hours at room temperature before filtering to remove any salt formed. The mixture was concentrated under reduced pressure and redissolved in CH₂Cl₂ (10 mL). The organic solution was washed with 5% aqueous NaHCO₃ (2 \times 5 mL) and saturated NaCl (5 mL). The organic layer was dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (1:4, MeOH:CH₂Cl₂) to give the final product **5** as a pale yellow oil (244 mg, 51%): R_f 0.25 (SiO₂, 1:4 MeOH:CH₂Cl₂); ν_{max} (neat)/cm⁻¹ 2931w, 1607m, 1507st, 1246st, 1133st, 1032st, 826st); δ_H (400 MHz, CDCl₃) 7.77-7.45 (2H, m, Ar-H), 7.37-7.28 (7H, m, Ar-H), 6.84 (4H, d, J 9.0 Hz), 3.81 (6H, s, OCH₃ \times 2), 3.07 (2H, t, J 7.0 Hz CH₂N), 2.68 (2H, t, J 7.0 Hz, CH₂O) 1.68-1.61 (2H, m, NCH₂CH₂), 1.48-1.28 (6H, m, NCH₂CH₂CH₂CH₂CH₂CH₂O); δ_C (100 MHz, CDCl₃) 158.7, 145.8, 137.2 & 86.1 (4 \times quaternary), 130.4, 128.6, 128.1, 126.9, 113.4 (5 \times CH), 63.8, 42.6, 34.2, 30.5, 27.2 & 26.6 (6 \times CH₂), 55.6 (1 \times CH₃); m/z (ES⁺) 118.1 (MH⁺-trityl). Literature data in agreement.⁵

1.8 Calculation of Slide Active Ester Content

- 1) Two slides of each type (3D hydrogel, 2D, Codelink and blank slides) were immersed in a solution of 4,4'-dimethoxytrityl-6-amino-1-hexanol **5** (0.36 mM, 0.15 mg/mL) dissolved in PBS buffer (25 mM PBS, 0.1 M NaCl, pH 8.5), which contained 8:2 water:DMF. 50 mL per slide was used.
- 2) The solution surrounding the slides was stirred for six hours at room temperature. The slides were removed and washed in distilled water (2 x 30 mins), and DMF (2 x 30 mins).
- 3) Slides were then placed in individual 50 mL Falcon tubes containing 30 mL of 30% v/v aqueous perchloric acid and 6 mL ethanol. The solution turned orange on addition of NHS ester slides. No colour change was seen with unfunctionalised glass.
- 4) Falcon tubes were placed horizontally and shaken for 12 hours on an Orbital Shaker S01 (Stuart Scientific) at a speed of 150 rpm.
- 5) The UV-visible spectrum was taken for every sample and the concentration of the 4,4'-dimethoxytrityl cation ($\epsilon_{498nm} = 70,000 \text{ M}^{-1}\text{cm}^{-1}$)⁷ in solution was calculated using the Beer-Lambert relationship (equation 1.1). The average concentration and average number of NHS groups per cm² for each slide was calculated.
- 6) For the 2D NHS ester slides, the calculated concentration was divided by two because both sides of the glass were functionalised.

Beer-Lambert Relationship

The concentration was calculated using the *Beer-Lambert* equation 1.1 where A = absorbance, ϵ = extinction coefficient ($\text{L mol}^{-1}\text{cm}^{-1}$), c = concentration (M) and l = path length of cell (1 cm).

$$A = \epsilon cl \quad (1.1)$$

1.9 Fluorescent Labelling of Proteins

1.9.1 Cy3 Labelling of Avidin⁶

Avidin (1 mg, 0.015 μmol) was dissolved in 1 mL PBS buffer (25 mM PBS, 0.1 M NaCl, pH 7.4). Bis *N'*-hydroxysuccinimide ester-Cy3 (0.488 mg, 0.51 μmol) was dissolved in 500 μL of PBS buffer (25 mM PBS, 0.1 M NaCl, pH 7.4). The protein solution was added to the dye and mixed thoroughly. The red reaction mixture was stirred for two hours in the dark at room temperature. The reaction was quenched with 100 μL Tris buffer (50 mM Tris, 0.1 M NaCl, pH 7.4) and stirred in the dark for a further 20 minutes.

1.9.2 Dialysis of Cy3-Avidin

The mixture of Cy3 labelled avidin and un-reacted dye was transferred to snakeskin dialysis tubing (Pierce Ltd., molecular weight cut off [MWCO] 3.5 kDa) and placed in 4 litres of Tris buffer (50 mM Tris, 0.1 M NaCl, pH 7.4). The container was covered with foil and stirred at 5 °C to dialyse out the un-reacted dye. The dialysis buffer was changed every 8-12 hours for 3 days. A sample was removed and the UV spectrum was obtained before dialysing one final time in Tris buffer over night. A further UV spectrum was obtained to ensure all unconjugated dye had been removed. This was done by comparing the dye/protein ratio from both UV spectrums. The dye/protein (D/P) ratio was calculated using Equation 1.2 and was equal to 4:1.

$$D : P = \frac{1.13 \times (A_{280nm})}{A_{280nm}(0.08 \times A_{560nm})} \quad (1.2)$$

1.10 Microarraying, Incubation and Visualisation

1.10.1 Microarraying

1. Biotin amine **6** was dissolved in DMF at a concentration 2.5 mM.
2. 1.25, 0.25 and 0.125 mM biotin amine **6** dilutions were made from the 2.5 mM stock solution.
3. The biotin alkyl control **7** was dissolved in DMF to 5 mM.
4. 15 μL of 2.5 mM, 1.25 mM, 0.25 mM, 0.125 mM biotin amine **6** and 5 mM biotin alkyl were pipetted into individual wells of a 384 well plate (7020 Genetix Ltd.).
5. The well plate was transferred to the source plate holder of a QArray_{LITE} microarrayer (Genetix Ltd.)
6. Four solid 300 μm pins (Genetix Ltd.) were loaded into the microarraying head. The pins were washed and dried in the microarray wash station with 95% ethanol using the diagnostic screen to ensure the lines were full of ethanol prior to printing.
7. The following settings were selected for printing using the Genetix QSoft software: four pin head layout; 384 well plate (7020 Genetix Ltd.) ordered by columns; 3x1" slides selected (4-pins, 12 fields maximum); a 5 x 5 printed array pattern per well; 0 blots; one sample per spot; one stamp per ink and one stamp per spot; 0 ms stamp time and 0 inking time; 0 μm print adjustment; 2 x 95% ethanol wash (5000ms wash and 5000ms dry).
8. 1 x 3D hydrogel slide, 1 x 2D NHS ester slide and 1 x Codelink NHS ester slide (GE Healthcare Ltd.) were placed in slide holders and the vacuum switched on to fix them in position during printing.
9. The printing was controlled using the computer software. Following printing, the slides were left in the chamber at 62.5% humidity for 12 hours.
10. Slides were transferred to a staining jar and washed with DMF (1 hour), 99% ethanol (1 hour) and distilled water (30 mins).
11. Slides were blocked with 50 mM ethanolamine in 25 mM PBS buffer pH 8.5 with stirring for 30 minutes.
12. Slides were rinsed with distilled water, dried under a stream of nitrogen before incubation with Cy3 labelled Avidin.

13. A data tracking file (.gal) was created containing details of the print pattern and the contents of each spot.

1.10.2 Protein Incubation

1. 200 μL of Cy3-avidin (at a concentration of 0.02 mg/mL) was pipetted onto PC200 cover wells (Sigma).
2. Printed slides were incubated by inverting the slide over the well. The slides were incubated for two hours in the dark at room temperature.
3. Following incubation, slides were carefully separated from the well using a scalpel.
4. Slides were transferred to a slide washer rack (Genetix Ltd.) and immersed vertically in 600 mL of Tris Tween buffer (50 mM Tris, 0.1 M NaCl, 0.5% Tween20, pH 7.4) within a 1 L beaker, which was stirred for 15 minutes whilst covered in foil.
5. The buffer was replaced with Tris buffer (pH 7.4, 50 mM Tris, 0.1 M NaCl) and stirred for a further 15 mins. The buffer was replaced and stirred for a further 15 mins.
6. The slide rack was removed and all slides were rinsed thoroughly with distilled water to remove any salts from the slide surface.
7. The slide rack was transferred to a dry 1 L beaker, and the slides were dried under nitrogen whilst covered in foil to protect against photo-quenching.
8. Dried slides were stored in a slide box ready to be scanned.

1.10.3 Visualisation

Dried slides were scanned for the biotin-avidin interaction using an aQuire scanner and QScan software (Genetix Ltd.). Slides were scanned using the Cy3 setting (at 35% PMT) with 0 μm focus adjustment at a resolution of 5 μm . The data tracking .gal file created was used to provide an overlay of blocks containing contents of individual spots. Individual blocks were aligned roughly over the fluorescent array before aligning them automatically using the QSoft program. Any individual spots misaligned were manually adjusted into the correct position. Statistics were generated for the background corrected Cy3 total intensity and the Cy3 SNR (signal-to-noise ratio).

1.11 AFM Measurement of Gel Slide Thickness

1. A Veeco atomic force microscope (AFM) was used with Nanoscope V5 AFM software to obtain surface data information on vacuum desiccator stored 3D slides. AFM tips (Olympus cantilever OMCL-AC160TS) were obtained from Atomic Force.
2. A clean scapel was used to scratch the surface of a dried gel. The AFM in tapping mode was used to generate a surface image, which was used to measure the step height between the glass and gel layer. Tapping mode settings used: scan size 5-10 μm , scan rate 0.3 Hz, tip velocity 6 $\mu\text{m/s}$, 256 samples per line, Z limit 5.291 μm , sample clearance 1000 μm , SPM (scanning probe microscope) safety limit 125 μm .
3. The surface image obtained was flattened using the Nanoscope software. The Section command was used to obtain three point to point 2D section plots, from which the step height was calculated, averaged and the standard deviation (SD) obtained. This was repeated for with another slide, in order to obtain an average film thickness between two slides (A and B) of 470 nm. See following page for AFM data.

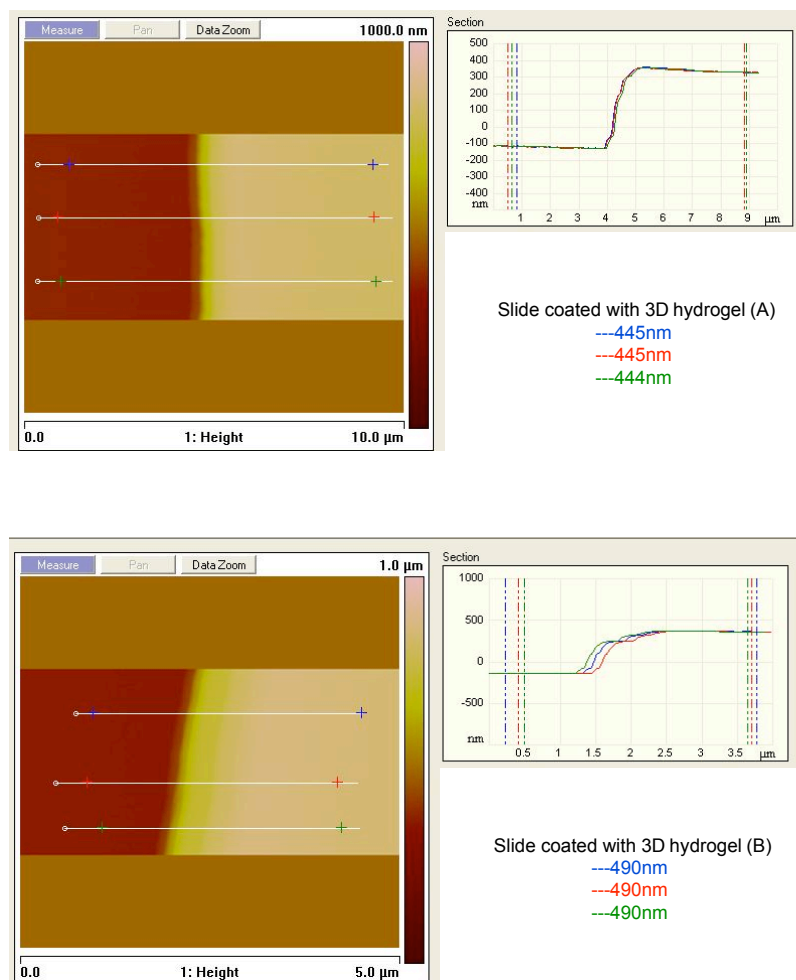


Figure 1.1: AFM image of slides A and B coated with the 3D polymer gel. Three cross-sectional measurements were made per slide to give an average of 470 nm over two slides.

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