3D small-molecule microarrays†

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A PEG based 3D hydrogel slide was developed specifically for small-molecule microarraying purposes, which displayed improved loading capacity, signal sensitivity and spot morphology compared with a commercially available slide and comparative 2D slide.

Microarray technology has established itself as a versatile tool for high throughput parallel investigation of molecular interactions. This has included complementary oligonucleotide hybridisations for gene expression profiling,[1] protein–protein interactions[2] and small-molecule–protein interactions.[3] The latter are investigated using small-molecule microarrays, in which small molecules, immobilised as spatially discrete spots on a solid support, are probed with a fluorescently labelled target protein. In this respect small-molecule microarrays have potential as a high throughput screening tool for the discovery of drug candidates or biological probes.[3] The detection of small-molecule–protein interactions is a significant challenge using small-molecule microarrays, because the interactions are often weak with a dissociation constant in the millimolar range. As a result there is a requirement for microarray slides with improved sensitivity for their detection.

Traditionally microarray slides are functionalised with a relatively planar 2D surface for immobilisation purposes. However 2D slides are often associated with low loading capacities and weak signal detection.[4] Slides that contain a 3D layer offer considerable advantages in terms of a higher loading capacity. Polyacrylamide gel pads have been developed for DNA and protein microarrays,[5] and have been commercialised by Perkin-Elmer (Hydrogel™ slides).[6] Slides coated with a polymer containing active esters for the covalent immobilisation of amines are also available commercially (Codelink™ slides).[7]

We envisaged developing a 3D polyethylene glycol (PEG) based microarray platform focused at the enhancement of small-molecule–protein detection by increased small-molecule loading capacity coupled with a reduction in non-specific interactions. PEG is made up of repeating ethylene glycol units $(CH_2CH_2O)_n$ and is an incredibly versatile material with a plethora of chemical and biochemical uses. It has been used to generate PEG based acrylamide resins for solid supported chemical synthesis,[8a] and also to generate PEG based slides for FRET based assays of peptides.[8b] PEG based surfaces offer a number of advantages for small-molecule microarray purposes. Firstly PEG reduces the adsorption of proteins at interfaces, which reduces non-specific interactions.[8c] Secondly PEG is amphiphilic, and therefore is an ideal immobilisation material for small-molecule microarray experiments, which require immobilisation of small molecules using organic solvents and protein incubation in aqueous buffer. 3D hydrogel slides were made as described in Scheme 1. Ordinary glass microscope slides were pre-functionalised with an acrylic ester silane before treatment with the polymerisation mixture containing the acrylamide PEG cross-linker **1**,[8a] the hydroxysuccinimide (NHS) active ester monomer unit **2**,[9] the co-monomer **3**[10] and the acetophenone UV radical initiator **4**. The polymerisation mixture was sandwiched with a hydrophobic cover slide, and polymerised upon irradiation with UV light (λ = 254 nm). The cover slide was removed to reveal an apparent uniform gel layer, which was washed, dried and analysed for slide thickness (Fig. 1) using an atomic force microscope (AFM). The average gel thickness across two different slides was calculated to be 470 nm.

For comparison purposes, 2D NHS ester slides were made according to the literature procedure[13] and commercial Codelink™ slides were purchased. In order to compare loading levels with the 3D hydrogel slide, the amine **5** was synthesised, which contains an acid cleavable dimethoxytrityl group (DMT).[11] Upon immobilisation of **5**, slides were treated with perchloric acid to generate the DMT cation in solution (Scheme 2), whose concentration was calculated using UV-Vis spectroscopy, from which the average number of NHS ester groups per cm^2 was calculated for each slide. The average number of NHS active ester groups on the 3D hydrogel slide was 7.3×10^{16} cm^{-2}, which was over an order of magnitude greater than the Codelink™ capacity (2.5×10^{15} cm^{-2}), which in turn was more than twice the 2D slide capacity (1.2×10^{15} cm^{-2}). We envisaged that the higher loading capacity of the 3D hydrogel slides would have a significant influence on the detected intensity level of fluorescence for a given small-molecule–protein interaction.

A standard small-molecule microarray experiment using amino-modified biotin as the small molecule and Cy3-labelled avidin as the protein was used to compare slides (Fig. 2A). Biotin-amine **6** at 2.5, 1.25, 0.25 and 0.125 mM, in addition to a biotin based negative control **7** at 5 mM, were printed onto slides using a commercial microarrayer (Genetix QArray). Printed slides were washed, incubated with Cy3 labelled avidin and scanned (dry) for Cy3 fluorescence shown in Fig. 2B. Slides were evaluated in terms of the following factors: how strong the signal was, the signal-to-noise ratio (SNR) and the spot morphology. The background corrected Cy3 intensity was plotted against the concentration of biotin-amine **6** using a log scale (Fig. 2C). All three slides displayed a linear

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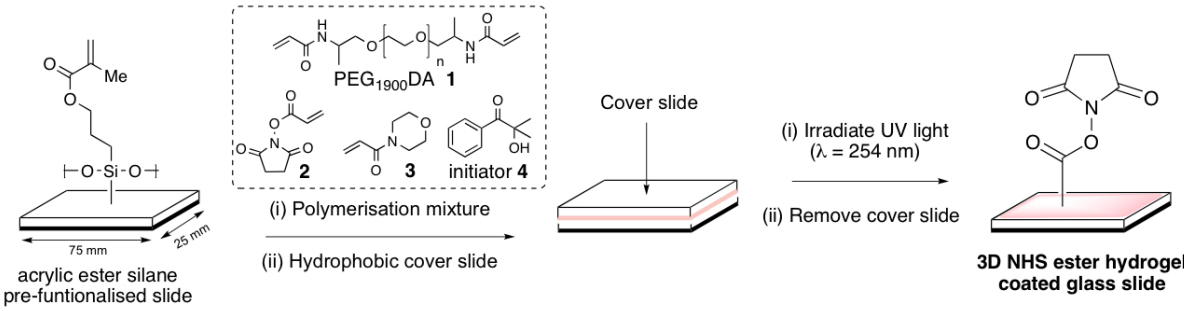


Scheme 1 Production of 3D hydrogel slides. **1** = bis-acrylamidoprop-1-yl-PEG$_{1900}$, **2** = acryloyloxysuccinimide, **3** = acryloylmorpholine and **4** = hydroxy-2-methylpropiophenone. Pre-functionalised glass slide generated by treatment of cleaned slide with bind-silane solution (methylacryloxypropyltrimethoxysilane in acidic ethanol–water mixture). The pre-polymerisation mixture, which was optimised in terms of loading capacity and microarray performance, consisted of 3% each of the cross-linker **1**, monomer **2**, co-monomer **3** and 1% initiator **4** in DMF. The hydrophobic slide was used to cover the polymerisation mixture before irradiation with UV light.

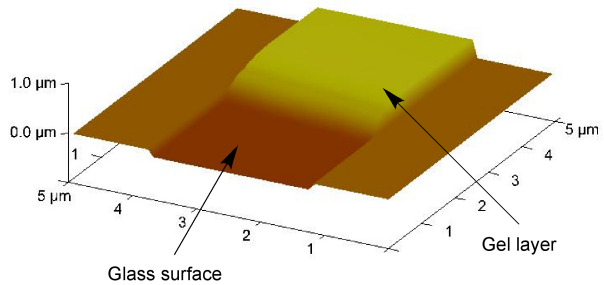


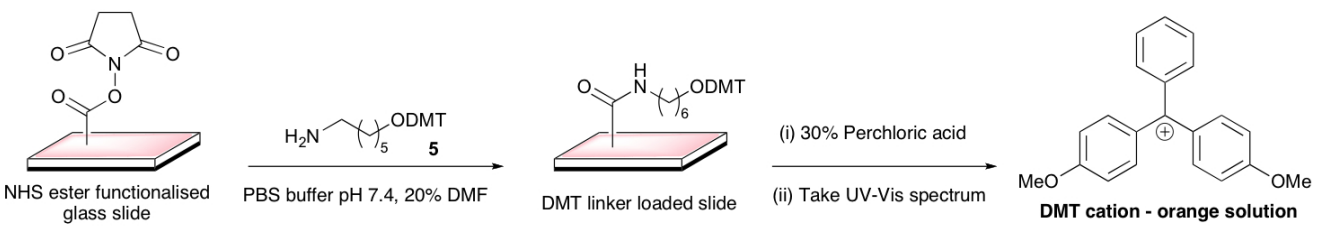
Fig. 1 AFM 3D surface image of a 3D hydrogel slide, where the gel had been removed from part of slide by a scratch test. Average gel thickness across two different slides = 470 nm, based on average of three different cross-sectional measurements per slide (490 and 450 nm). See ESI† for further details.

relationship between fluorescence and concentration. The 3D hydrogel slide produced on average, a six-fold higher Cy3 intensity over the Codelink™ slide, essentially because of the 3D hydrogels superior loading capacity. The ability to detect small-molecule–protein interactions at a higher fluorescent signal is particularly relevant to their discovery given that the interactions are often much weaker compared with complementary oligonucleotide hybridisations and protein–protein interactions. The requirement to scan the 2D slide at a higher gain setting demonstrates its lower loading capacity compared to the other two slides. The negative control biotin **7** showed little sign of non-specific retention across all three slides.

The SNR is defined as the background corrected fluorescent signal over the standard deviation of the background and demonstrates how obtrusive the background noise is to the fluorescent signal for a particular slide. The 3D hydrogel slides produced the highest average SNR across the concentration range of **6** printed, with a SNR of 24, compared to the Codelink™ and 2D slides, which had values of 17 and 7, respectively. Improvements in the SNR are likely in part due to the presence of PEG in the hydrogel, which reduces the non-specific adsorption of proteins.[8c] It should be noted that at 2.5 mM **6**, the 3D hydrogel slide generated particularly large spots, which can be spaced further apart using a larger pitch distance when printing. Alternatively smaller microarray pins can be used to reduce the size of spots.

The 3D hydrogel slide also produced a more consistent spot morphology in terms of both spot size and shape compared with the 2D and Codelink™ slides. In particular the 3D hydrogel slide produced a lower variation in spot diameter with a coefficient of variation (CV) range between 2 and 4%, compared with 3–8% and 2–6% for the Codelink™ and 2D slides, respectively.[12] Regular spot morphology is particularly important for accurate comparison of different spots within a large-scale microarray screen.

In summary, we have developed a PEG based 3D hydrogel microarray slide, which contains active esters for the immobilisation of small-molecule amines. Loading studies revealed our 3D hydrogel slide had over an order of magnitude greater loading capacity compared to a commercial Codelink™ slide and a comparative 2D slide. A standard small-molecule microarray experiment also revealed that the 3D slides produced a significantly higher fluorescent signal and improved spot morphology across the concentration range of the small-molecule biotin printed. We envisage that these key advantages will enable us to use our 3D small-molecule microarray



Scheme 2 NHS ester slide loading capacity experiment using the dimethoxytrityl (DMT) linker **5**.[11] Following immobilisation of **5**, slides were treated with perchloric acid, which generated a deep orange colour corresponding to the cleaved DMT cation, whose concentration was calculated using UV-Vis spectroscopy (ε_{498} = 70 000 M^{-1} cm^{-1}). The average number of NHS ester groups for each type of slide was calculated across two replicate slides: 3D hydrogel = 7.3 × 10^{16} cm^{-2}, Codelink™ = 2.5 × 10^{15} cm^{-2} and 2D = 1.2 × 10^{15} cm^{-2}.

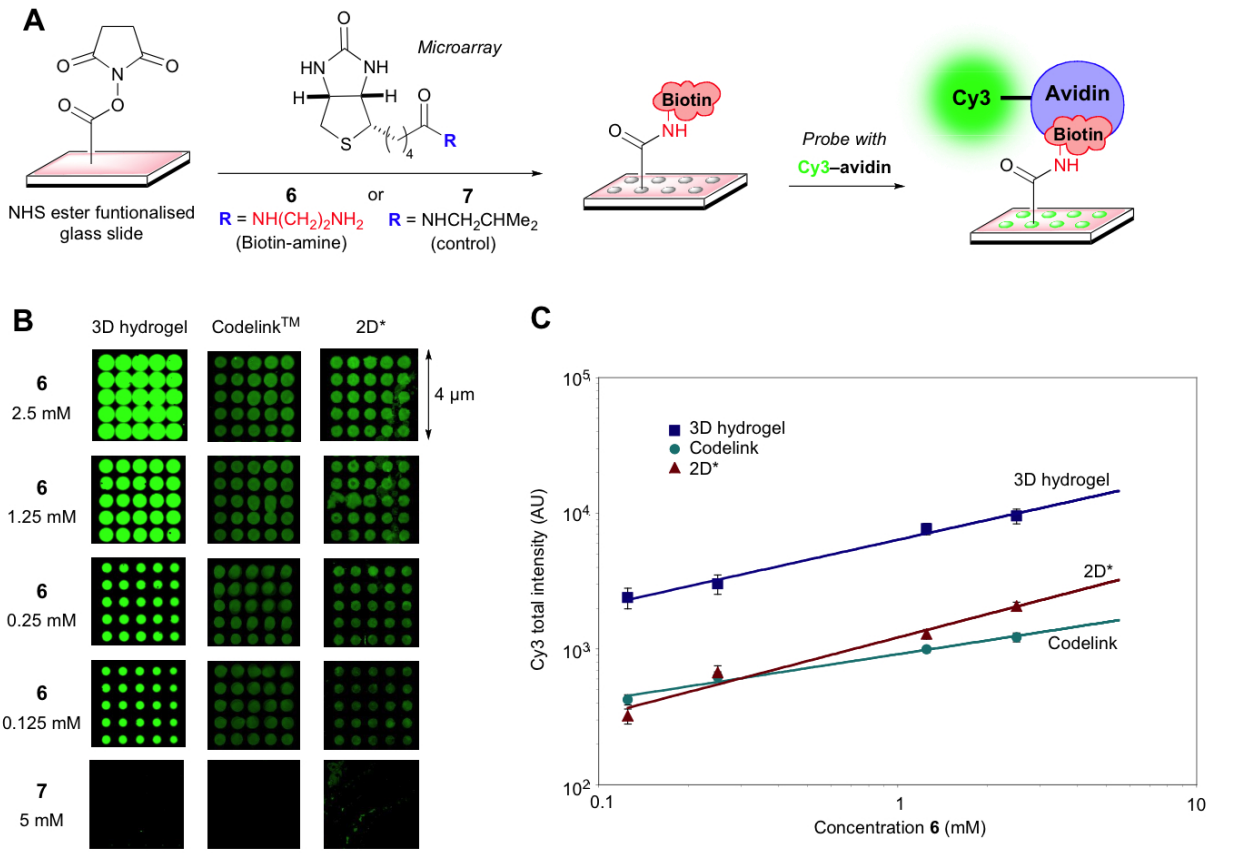


Fig. 2 (A) Small-molecule microarray experimental schematic in which biotin-amine **6** at 0.125, 0.25 and 1.25 mM and the biotin negative control **7** at 5 mM were both printed onto the three NHS ester slides prior to treatment with Cy3 labelled avidin. (B) Cy3 scanned images of the 3D hydrogel (dry), Codelink™ and 2D slides. *2D slide did not display fluorescence at standard scanning settings so was rescanned at 40% PMT.[12] (C) The background corrected Cy3 total intensity was plotted against the concentration of **6**, using the log scale. Values were averaged over 25 spots and error bars correspond to the standard deviation.

focused platform to discover new small-molecule–protein interactions, which would be beyond the detection capacity of traditional microarray platforms.

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Notes and references

1 M. Schena, D. Shalon, R. W. Davis and P. O. Brown, *Science*, 1995, **270**, 467–470.

2 G. MacBeath and S. L. Schreiber, *Science*, 2000, **289**, 1760–1763.

3 (*a*) G. MacBeath, A. N. Koehler and S. L. Schreiber, *J. Am. Chem. Soc.*, 1999, **121**, 7967–7968; (*b*) R. L. Nicholson, M. Ladlow and D. R. Spring, *Chem. Commun.*, 2007, 3906–3908. For recent reviews see; (*c*) R. L. Nicholson, M. Welch, M. Ladlow and D. R. Spring, *ACS Chem. Biol.*, 2007, **2**, 24–30; (*d*) A. J. Vegas, J. H. Fuller and A. N. Koehler, *Chem. Soc. Rev.*, 2008, **37**, 1385–1394.

4 D. W. Grainger, C. H. Greef, P. Gong and M. J. Lochhead, *Methods Mol. Biol. (Totowa, N. J.)*, 2007, **381**, 37–57.

5 (*a*) E. Timofeev, S. V. Kochetkova, A. D. Mirzabekov and V. L. Florentiev, *Nucleic Acids Res.*, 1996, **24**, 3142–3148; (*b*) J. Zlatanova and A. Mirzabekov, *Methods Mol. Biol. (Totowa, N. J.)*, 2001, **170**, 17–38; (*c*) P. Arenkov, A. Kukhtin, A. Gemmell, S. Voloshchuk, V. Chupeeva and A. Mirzabekov, *Anal. Biochem.*, 2000, **278**, 123–131.

6 J. C. Miller, H. Zhou, J. Kwekel, R. Cavallo, J. Burke, E. B. Butler, B. S. Teh and B. B. Haab, *Proteomics*, 2003, **3**, 56–63.

7 R. Ramakrishnan, D. Dorris, A. Lublinsky, A. Nguyen, M. Domanus, A. Prokhorova, L. Gieser, E. Touma, R. Lockner, M. Tata, X. Zhu, M. Patterson, R. Shippy, T. J. Sendera and A. Mazumder, *Nucleic Acids Res.*, 2002, **30**, 30.

8 (*a*) M. Meldal, *Tetrahedron Lett.*, 1992, **33**, 3077–3080; (*b*) M. Zourob, J. E. Gough and R. V. Ulijn, *Adv. Mater.*, 2006, **18**, 655–659; (*c*) Y. S. Lin, V. Hlady and C. G. Gölander, *Colloids Surf., B*, 1994, **3**, 49–62.

9 M. Mammen, G. Dahmann and G. M. Whitesides, *J. Med. Chem.*, 1995, **38**, 4179–4190.

10 F. D'Agosto, M. T. Charreyre, F. Melis, B. Mandrand and C. Pichot, *J. Appl. Polym. Sci.*, 2003, **88**, 1808–1816.

11 A. Avino, R. Guimil Garcia, F. Albericio, M. Mann, M. Wilm, G. Neubauer and R. Eritja, *Bioorg. Med. Chem.*, 1996, **4**, 1649–1658.

12 Fluorescent scanning was performed using a Genetix aQuire scanner. 3D hydrogel and Codelink™ slides were scanned at 35% PMT (photomultiplier tube setting), 2D at 40% PMT. The coefficient of variation (CV) is the standard deviation expressed as % of the mean.

13 R. Benters, C. M. Niemeyer and D. Wohrle, *ChemBioChem*, 2001, **2**, 686–694.