

Fluorous tagged small molecule microarrays†

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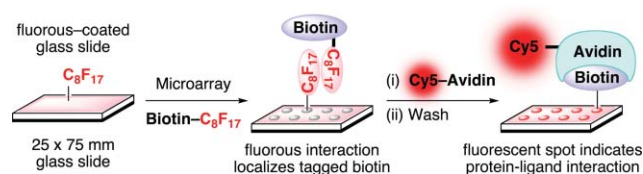
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The affinity fluorous interaction between fluorous tagged small molecules and a fluoroalkyl modified glass surface was shown to facilitate the detection of protein–ligand binding interactions in the fabrication and screening of small molecule microarrays.

Small molecules are valuable in the fields of medicine and biology to modulate and elucidate protein function rapidly, reversibly and conditionally with temporal and quantitative control.¹ Traditionally small molecules with desirable biological properties have been discovered through a time and resource intensive approach involving synthesis then screening of individual compounds. However, small molecule microarrays have the potential to act as a smaller, faster and cheaper alternative to traditional methods. Currently available technology typically requires inclusion of a reactive functional group in the small molecule to facilitate covalent immobilization onto the glass substrate.² Whilst successful in the identification of novel small molecule–protein binding partners,³ this approach can involve extensive protecting group manipulation and precludes the synthesis of compounds containing incompatible functional groups. Alternative strategies for microarray fabrication, which exploit non-covalent interactions between the arrayed compound and the substrate, have also been developed.⁴ One method, demonstrated by Pohl and co-workers in the preparation and screening of carbohydrate microarrays,⁵ utilizes the highly specific fluorous affinity interaction between compounds tagged with a ‘light’ fluorous alkyl chain, such as C₈F₁₇, and fluoroalkyl modified glass slides. This approach has two key benefits: firstly, the polyfluorocarbon chain is chemically inert and compatible with a wide range of functional groups; and, secondly, the fluorous tag enables solution phase parallel synthesis with the generic purification of product from reagents by fluorous solid-phase extraction (SPE), reverse fluorous SPE or liquid–liquid extraction.⁶ In this paper, we describe a light polyfluorocarbon immobilization tag suitable for arraying and screening drug-like small molecules, such as biotin, onto fluorous-coated glass slides (Scheme 1).⁷

In our initial studies the suitability of fluorous slides for the detection of protein–ligand interactions was assessed using the small molecule–protein partners, biotin and avidin. Biotin was derivatized with three polyfluorocarbon tags to enable site specific immobilization of the compounds (**1–3**, Fig. 1) onto the fluorous-coated substrate. The compounds were arrayed onto commercially



Scheme 1 The preparation of fluorous tagged small molecule microarrays, which exploit the fluorous affinity interaction between the tagged small molecule (fluorous tagged biotin shown) and the fluoroalkyl modified slide.

available⁸ fluoroalkyl-coated slides *via* robotic pin contact printing. After drying the slides were incubated with Cy5-labelled avidin, washed with pH 7.4 phosphate buffer containing 1% detergent (Tween-20) and scanned for fluorescence. Positive interactions between biotin and avidin are revealed as false-coloured red spots (Fig. 1).

Assessment of the slides following treatment with the protein indicated that all of the fluorous tagged biotin compounds (**1–3**) can withstand the 30 minute incubation period with the protein and the subsequent washing steps with the detergent-containing buffer solution to varying extents. The biotin amide **3** gave superior results in terms of assay consistency, signal intensity, spot morphology and spot size when compared to the biotin derivatives **1** and **2**. The unsatisfactory results obtained for **1** are believed to

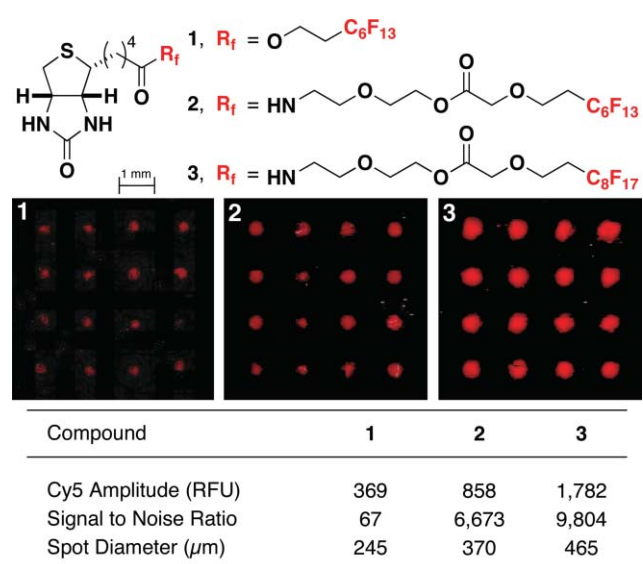


Fig. 1 Detection and assessment of the interaction between Cy5-labelled avidin and biotin derivatives **1**, **2** and **3** immobilized on a fluorous-coated glass surface. Values shown refer to average values for the representative images (4 × 4 blocks) shown. RFU = relative fluorescence units.

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result from the highly hydrophobic nature of this compound which inhibits the interaction with the protein. The presence of the polyethylene glycol spacer between the biotin and the fluororous tag modifies the properties of the molecule increasing the solubility in water whilst retaining the hydrophobic properties necessary for immobilization on the slide. However, there appears to be a reasonably fine balance operating as the less favourable assay results obtained for **2** relative to **3** are thought to be due to the partial removal of the biotin amide (**2**) in the incubation and washing steps. The shorter fluororous chain in **2** (C_6F_{13}) compared to **3** (C_8F_{17}), resulting in a lower fluororous interaction,⁹ is likely to be primarily responsible for this. As the C_8F_{17} polyfluorocarbon tag gave the most consistent results this compound (**3**) was used in further experiments. The utility of using this methodology to identify biotin–avidin interactions was demonstrated by arraying a small collection of fluororous tagged low molecular weight amides and controls onto the fluororous slides and probing with fluorescently-labelled avidin. The small molecules were arrayed onto the slides as 10 mM solutions¹⁰ in dimethylformamide and allowed to dry.[†] Each slide was assessed visually prior to treatment with the protein. Good spot morphology (small discrete spots with no visible cross contamination between probe sites) was observed for the fluororous tagged compounds. In contrast, untagged biotin (**A**), which had been printed as a control, showed very poor spot morphology with visible “bleeding” between the array sites. The slides were then probed with Cy5-labelled avidin and scanned for fluorescence (Fig. 2).

While fluororous tagged biotin **3** was selectively detected by the protein (Fig. 2), observation of the biotin–avidin interaction proved somewhat capricious between different array sites on the slides. We hypothesized that this is a result of the hydrophobic nature of the slide surface which prevents effective movement of the protein across the substrate surface. Treatment of the slides with 3% bovine serum albumin (BSA) solution prior to incubation with the avidin overcomes this difficulty, giving more consistent results in terms of the spot morphology, signal intensity and assay consistency (Fig. 3).

The slides can be cleaned for reuse by washing sequentially with methanol and dichloromethane. This successfully removes the arrayed small molecules and residual protein; a scan of the cleaned slide shows no fluorescence at either the Cy3 or Cy5 frequencies. The slides were then reprinted with the small molecule collection (A–L) and probed with Cy5-labelled avidin. The biotin–avidin

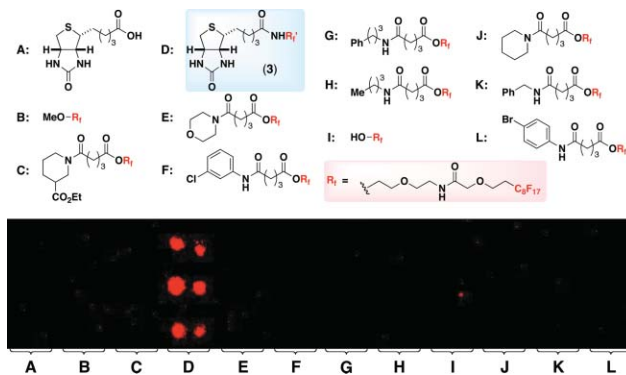
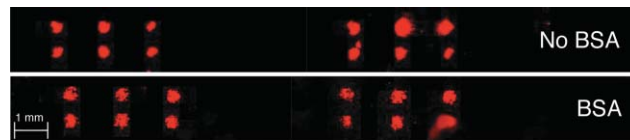


Fig. 2 Specific detection of fluororous tagged biotin–avidin interaction within a collection of microarrayed small molecules (A–L).



Slide Treatment	No BSA		BSA	
	Cy5 Amplitude (RFU)	1394	7339	947
Spot Diameter (μm)	350	440	380	390

Fig. 3 Comparison of slides treated with either 3% BSA solution or no blocking protein prior to incubation with Cy5-labelled avidin. Average values for each set of six spots (3×2 block) shown. BSA = bovine serum albumin; RFU = relative fluorescence units.

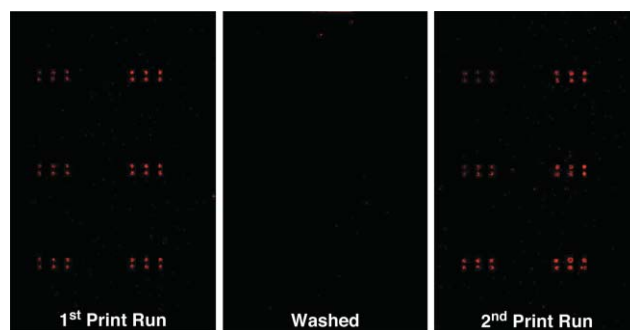


Fig. 4 Slide reuse. Slides can be washed and reused with no apparent deterioration in quality of results.

interaction was successfully observed with minimal background fluorescence and non-specific interactions between the protein and the other compounds (Fig. 4).¹¹

In conclusion, we have shown that a fluororous interaction allows for the ready formation of drug-like small molecule microarrays which are compatible with the discovery of small molecule–protein interactions. The non-covalent fluororous interaction between the tagged small molecule and the slide surface minimizes the need for protecting group strategies currently required when covalent immobilization is employed. Since fluororous tagged small molecules are conveniently prepared using solution phase parallel synthesis techniques, this high throughput, miniaturized method of screening should prove especially valuable.

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Notes and references

[†] *General procedure:* Compounds were printed as 10 mM solutions in dimethylformamide onto fluororous glass slides by robotic pin contact printing using solid $300 \mu\text{m}$ pins at 70% humidity using a Genetix Qarray microarrayer. The slides were left standing on the microarrayer bed in a humid environment for 18 h, then soaked in 3% bovine serum albumin solution if required. The slides were briefly washed with water then treated with fluorescently labelled protein ($25 \mu\text{g}$ of Cy5-labelled avidin dissolved in $225 \mu\text{L}$ of H_2O). The slides were incubated at room temperature in a humid, dark environment for one hour then washed in pH 7.4 phosphate buffer with 1% Tween ($\times 3$) and distilled water ($\times 2$) and dried (N_2 atmosphere). The dried slides were scanned at 50% photomultiplier tube

gain setting at the Cy5 wavelength using a confocal scanner (Genetix Qscan).

- 1 S. L. Schreiber, *Nat. Chem. Biol.*, 2005, **1**, 64–66; D. P. Walsh and Y.-T. Chang, *Chem. Rev.*, 2006, **106**, 2476–2530; D. R. Spring, *Chem. Soc. Rev.*, 2005, **34**, 472–482.
- 2 For recent reviews see: J. L. Duffner, P. A. Clemons and A. N. Koehler, *Curr. Opin. Chem. Biol.*, 2007, **11**, 74–82; X. G. He, G. Gerona-Navarro and S. R. Jaffrey, *J. Pharmacol. Exp. Ther.*, 2005, **313**, 1–7; M. Uttamchandani, D. P. Walsh, S. Q. Yao and Y.-T. Chang, *Curr. Opin. Chem. Biol.*, 2005, **9**, 4–13.
- 3 For example see: S. E. Tully, M. Rawat and L. Hseih-Wilson, *J. Am. Chem. Soc.*, 2006, **128**, 7740–7741; M. Uttamchandani, D. P. Walsh, S. M. Khersonsky, X. Huang, S. Q. Yao and Y.-T. Chang, *J. Comb. Chem.*, 2004, **6**, 862–868; A. N. Koehler, A. F. Shamji and S. L. Schreiber, *J. Am. Chem. Soc.*, 2003, **125**, 8420–8421; F. G. Kuruvilla, A. F. Shamji, S. M. Sternson, P. J. Hergenrother and S. L. Schreiber, *Nature*, 2002, **416**, 653–657.
- 4 J. J. Diaz-Mochón, L. Bialy, L. Keinicke and M. Bradley, *Chem. Commun.*, 2005, 1384–1386; H. D. Urbina, F. Debaene, B. Jost, C. Bole-Feysot, D. E. Mason, P. Kuzmic, J. L. Harris and N. Winssinger, *ChemBioChem*, 2006, **7**, 1790–1797.
- 5 K.-S. Ko, F. A. Jaipuri and N. L. Pohl, *J. Am. Chem. Soc.*, 2005, **127**, 13162–13163; S. K. Mamidyala, K.-S. Ko, F. A. Jaipuri, G. Park and N. L. Pohl, *J. Fluorine Chem.*, 2006, **127**, 571–579.
- 6 For reviews see: A. Studer, S. Haddida, R. Ferritto, S.-Y. Kim, P. Jeger, P. Wipf and D. P. Curran, *Science*, 1997, **275**, 823–826; D. P. Curran, in *The Handbook of Fluorous Chemistry*, ed. J. A. Gladysz, D. P. Curran and I. T. Horvath, Wiley-VCH, Weinheim, 2004, pp. 101–155; W. Zhang, *Chem. Rev.*, 2004, **104**, 2531–2556; D. P. Curran, *Aldrichimica Acta*, 2006, **39**, 3–9.
- 7 During the publication of this paper similar research has appeared in press: A. J. Vegas, J. E. Bradner, W. Tang, O. M. McPherson, E. F. Greenberg, A. N. Koehler and S. L. Schreiber, Fluorous-Based Small-Molecule Microarrays for the Discovery of Histone Deacetylase Inhibitors, *Angew. Chem.*, in press.
- 8 Fluorous modified slides; Fluorous Technologies, Inc., 970 William Pitt Way, Pittsburgh, PA 15238, USA.
- 9 C. S. Wilcox, V. Gudipati, H. Lu, S. Turkyilmaz and D. P. Curran, *Angew. Chem., Int. Ed.*, 2005, **44**, 6938–6940.
- 10 Lower concentrations of stock solutions have been used (down to 250 μ M) without deterioration in quality of results.
- 11 Slides have been reused five times without noticeable deterioration in quality of results, although the upper limit of reuse may be much higher than this.

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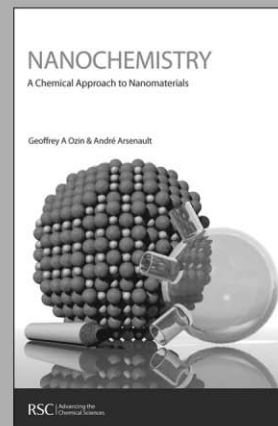
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