Microarrays

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Fluorous Tags Catching on Microarrays

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Microarrays provide a convenient way to probe biomolecular interactions with a minimum amount of sample and therefore have seen wide use in screens of primarily immobilized nucleic acids and proteins/peptides. Arrays of small molecules such as natural products, including carbohydrates, and druglike molecules have been slower to develop, in part because access is challenging to the compounds themselves with handles that allow specific attachment to an array surface. Most methods rely on the intrinsic reactivity of a nucleophilic group in the small molecule for covalent modification with reactive electrophiles on the microarray slide surface.[1] Of course, multiple nucleophiles are common in small molecules and therefore the exact mode of attachment is unknown. A unique functional handle can be included in the initial design and execution of synthetic compound libraries, but this strategy adds complexity.

One solution to this problem of selectively attaching small molecules to an array surface without introducing new reactive functional groups was reported in 2005 and relies on use of a fluorocarbon tag more commonly used to simplify purification steps (Figure 1).[2] Fluorocarbons make convenient tags for the separation of intermediates in small-molecule library synthesis.[3] The fluorous tags themselves are relatively unreactive, are invisible in proton NMR spectra, and phase separate from water and organic solvents for simple liquid or solid-phase extraction. In fact, unlike hydrocarbon tags, which require up to 36 carbon atoms for reliable solid-phase extraction on C18 columns,[4] fluorous tags of only eight carbon atoms are adequate for extraction on fluorous silica gel by use of solvophobic interactions. The initial report in 2005 demonstrated that this fluorous solvophobic effect was also sufficiently strong to adhere fluorous-tagged monosaccharides to a C_8F_{17}-modified glass surface in defined locations. Unprotected carbohydrate ligands attached to a C_8F_{17} tag through a butenediol spacer were spotted on the modified glass slides and then screened with fluorescently labeled carbohydrate-binding proteins. Even low amounts of a hydrocarbon-based detergent often found in bioassay reports were tolerated in the screening buffer. This initial work was then extended to show that disaccharides and charged sugars could also be probed with plant lectins by using such a fluorous-based strategy.[5]

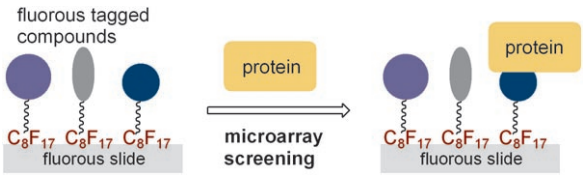

Figure 1. A fluorous small-molecule microarray formation strategy relies on noncovalent forces to surface pattern fluorous-tagged compounds in defined locations on fluorous-derivatized glass slides. Incubation of the slides in aqueous buffers containing proteins, either directly fluorescently labeled or subsequently bound with fluorescently labeled antibodies, allows the detection of small molecule/protein interactions upon fluorescence scanning.

The original concept of fluorous-based small-molecule microarrays was demonstrated with unprotected carbohydrate ligands. Now new work[6] shows that this approach is not limited to ligands as hydrophilic as carbohydrates (Figure 2). The first small-molecule microarray for the screening of inhibitors of histone deacetylases (HDAC) validates the use of noncovalent fluorous-based microarrays with more hydrophobic druglike molecules. Histone deacetylases are a group of zinc hydrolases that alter gene expression by hydrolysis of acetylated lysines on transcription regulatory proteins; inhibitors of these enzymes show promise in the treatment of cancer, neurodegenerative diseases, fibroproliferative disorders, inflammatory diseases, and viral and protozoal infections.[7] Inhibitors selective for one HDAC over another could help dissect the individual functions of these hydrolases in controlling gene expression. Vegas et al. screened a collection of HDAC inhibitors modified with fluorous tags to identify and compare HDAC inhibitors. The inhibitors were chosen with varying linker lengths, metal chelating groups, and known affinities to interrogate the fluorous-based microarray approach. The small-molecule array was printed in replicates and screened against HDAC2, the HDAC3/NCoR2 peptide complex, and HDAC8. The addition of a dye-labeled anti-pentaHis antibody allowed visualization of the binding interactions. The noncovalent attachment strategy was shown to tolerate the removal of unbound HDAC from the slide, subsequent incubation with antibodies, and several rinses before scanning with a fluorescent scanner. In fact, the fluorous arrays are even robust enough to be incubated with

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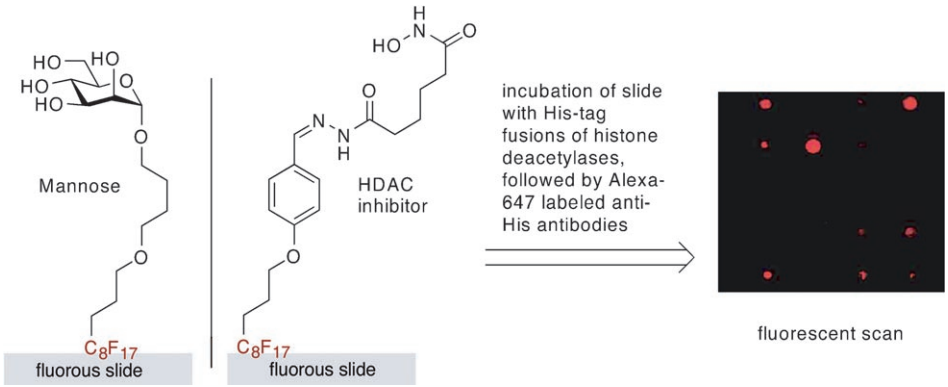

Figure 2. The fluorous microarray strategy was originally developed for the screening of hydrophilic carbohydrates. New work shows that more hydrophobic molecules such as histone deacetylase inhibitors can also be screened by noncovalent fluorous interactions.

soluble inhibitors for competition-binding experiments in the microarray format and to be used for the screening of whole-cell lysates and not just purified proteins. The authors report that the fluorous-based microarray allows the controlled display of the inhibitory functional groups with a low uniform background and great signal-to-noise ratios.

This new work also demonstrates that there is a strong correlation between HDAC inhibitors found by fluorous-based small-molecule microarrays and those found by solution-based biochemical assays and surface plasmon resonance (SPR) based screening. However, a few of the inhibitors did not work as well on the fluorous microarray surface as they did in a solution-based biochemical assay for HDAC activity. Interestingly, SPR experiments to determine the kinetics and thermodynamics of inhibitor binding showed that these particular inhibitors showed comparatively fast dissociation rates ($>0.1\ s^{-1}$). As often not only high affinity, but also low dissociation rates are desirable in optimizing drug–target interactions, fluorous-based small-molecule microarrays have the potential to filter out hits on the basis of affinities as well as dissociation rates.

The HDAC inhibitor study also highlights key practical issues in the successful generation of microarrays with more-hydrophobic compounds. Washing protocols during microarray printing are crucial to prevent cross-contamination, especially with less-hydrophilic compounds. Printing pins were sonicated in dimethylformamide prior to array printing and then washed five times in the same solvent with vacuum drying between different samples in the printing runs.

Interestingly, another group at the University of Cambridge has also recently found noncovalent fluorous interactions to be valuable in the generation of small molecule microarrays.[8] In that work, fluorous-tagged biotin molecules were printed onto fluorous glass slides and screened with dye-labeled avidin (Figure 3). As would be expected in an array strategy that relies on noncovalent solvophobic interactions, C_8F_{17}-modified biotin gave more consistent results and better spot morphology and size than the more expensive, shorter C_6F_{13}-tagged analogue. For comparison, untagged biotin was also spotted on the fluorous surface and visibly bled between array spots. The fluorous tag is unquestionably necessary for

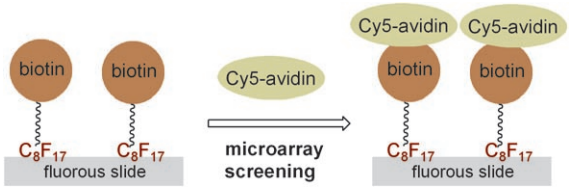

Figure 3. A general hydrophobic effect is not sufficient for the neat spot morphologies seen upon spotting of fluorous slides. Biotin tagged with a C_8F_{17} label for arraying and then visualized after binding to Cy5-labeled avidin exhibited reliable spots, whereas untagged biotin spotted on the fluorous slides obviously bled between array spots.

surface patterning of small molecules into discrete spots required for microarray screening; a general hydrophobic effect will not suffice. The authors also establish the unique ability of fluorous-derivatized slides to be reused at least five times by simple washing with organic solvents. The reprinted slides displayed little background fluorescence and nonspecific interactions between avidin and other compounds not related to the structure of biotin.

Our understanding of the strength and utility of fluorous solvophobic effects is clearly still in its infancy. However, these noncovalent forces appear to be strong enough to reliably pattern and screen a range of molecules even in the presence of detergents and thereby make noncovalent small molecule microarray strategies more appealing. The recent demonstration that these noncovalent fluorous microrrays can also support quantitative binding experiments should further enhance the utility of fluorous-based screening approaches.[9]

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