

TALKING ABOUT AN SMM REVOLUTION?

The use of small-molecule micro-arrays is set to make a huge impact in drug discovery. Dr Nicholas Westwood and Stephen Patterson of St Andrews University, discuss its potential.



Author

Dr Nicholas J Westwood, School of Chemistry and Centre for Biomolecular Sciences at St Andrews University, in the UK, he graduated from Oxford University in 1992. Stephen Patterson has been a post-doctoral researcher at the Westwood laboratory for three years, and has developed a strong interest in chemical genetics.

DNA/RNA micro-array technology has established itself in the mainstream of post-genomic science. Customised chips are commercially available from a range of suppliers and impressive applications of the technology already exist. However, the techniques that are at the heart of micro-arraying have also inspired related technologies (see Figure 1), which are not yet as well developed, but may prove as influential. One of these technologies, the use of small-molecule micro-arrays (SMMs), could be key in revolutionising the drug discovery process.

Although important, the commercial availability of micro-arrays is not the only driver in the development of SMMs. This technology would be nowhere without the synthetic chemist. The ability to prepare high-quality, spatially arrayed, small molecule collections is integral to the SMM approach. It is the use of high throughput synthesis, coupled with the creativeness of the synthetic chemist, that delivers the small molecule collections that are being arrayed. In essence, the approach is a simple one involving four key issues:

1. Access to a small molecule collection, ideally with each member of the collection containing one key functional group used to fix it in position on the array (the collection must be in a format that is compatible with the micro-arraying technology used).
2. Selection of a solid support on which to array the small molecule collection.
3. Identification of a chemical technology that enables each small molecule from 1 to be linked to the selected solid support.
4. Selection of an assay method that can be used in conjunction with the array (for example, a means of identifying biomolecules) that bind to specific small molecules present in the array).

The details inherent in 1 and 4 fall outside the scope of this article and the reader is referred to one of the many review

articles on high throughput/diversity-oriented synthesis and micro-array technology. Instead, issues 2 and 3 are the focus.

SMM chemical ligation strategies

While cellulose sheets and polymer-based membranes have been used, it is the low intrinsic fluorescence properties of glass coupled with its mechanical strength and low cost that makes it the main support used to date. It is also relatively easy to functionalise a glass surface, resulting in a series of chemical ligation strategies for covalently attaching small molecules to the array. Early approaches from the research group of Stuart Schreiber at Harvard University, focused on the reaction between maleimide-functionalised glass slides and thiol containing small molecules (see Figure 2; 1). However, its overall applicability proved limited.

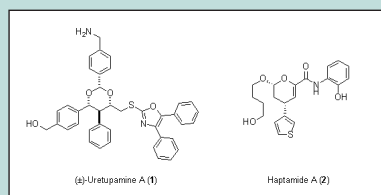
A key development in the Schreiber approach came with the second-generation arrays in which alcohol-containing, small molecules were reacted with chlorinated glass slides forming a stable silicon-oxygen linkage (see Figure 2; 2). This ingenious solution provided the necessary link between Schreiber's small molecule synthesis technology (which typically delivers small molecules containing an alcohol-functional group) and the ability to prepare SMMs. It is this combination of techniques from the Schreiber laboratory that has resulted in several of the successful applications of SMMs.

More recently, two alternative chemical technologies that bridge the gap between small molecule library generation and SMM preparation (see Figure 2; 3 and 4) have been reported. One from the Waldmann laboratory involves a chemoselective method of coupling azide-functionalised small molecules to phosphane-decorated glass slides (known as the Staudinger ligation). A second involves the selective reaction of epoxide-coated glass surfaces with small molecules containing a hydrazide functional group.

Other important players in the SMM field have used glyoxylyl-derivatised glass slides that enable peptides or small molecules to be attached through a thiazolidine ring or oxime formation respectively (see Figure 2; 5). German company, Graffinity Pharmaceuticals AG has developed a core technology based on gold-coated micro-structured glass slides in which small molecules are linked to the surface via a self-assembled monolayer of molecules that contain both a binding point to provide the link to the small molecule and a thiol to bind to the gold surface (see Figure 2; 6). The use of 1,3-dipolar cycloadditions, N-hydroxysuccinimide esters, Diels-Alder linking strategies and diazobenzylidene-derivatised glass slides have also been reported (see Figure 2; 7-10).

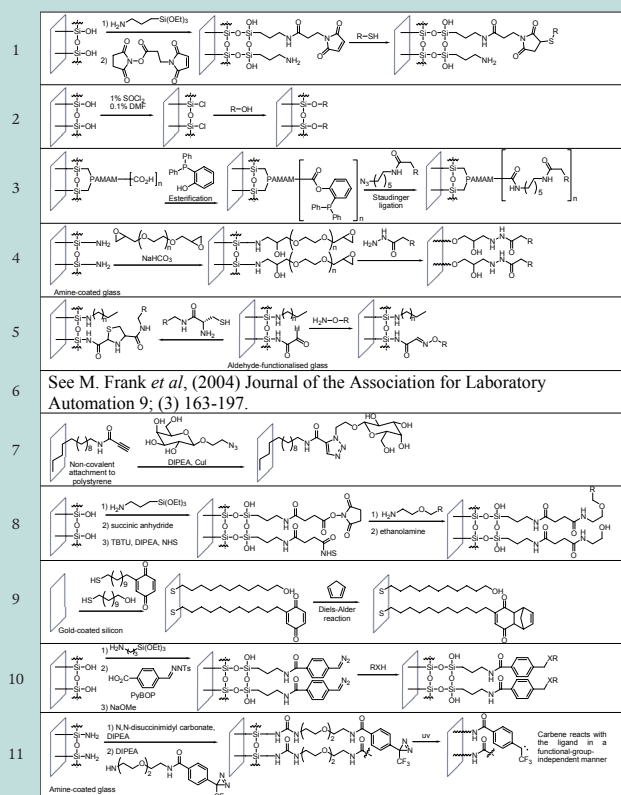
While the majority of the strategies place a high price on chemoselective attachment of the small molecule to the array with the aim of ensuring uniform presentation of the small molecule to a potential binding partner, a Japanese group has taken an alternative approach (see Figure 2; 11). UV irradiation of diazirin-coated glass slides in the presence of the small molecule results in initial formation of a very reactive glass-

Figure 1. Biologically relevant small molecules discovered using SMM technology



in the correct orientation for binding may result in high signal to noise ratios. David Spring, an academic in the School of Chemistry at Cambridge University is actively involved in developing SMM technology, who comments: 'One of the main challenges for science in this area is to ensure high sensitivity and signal to noise ratios.'

Figure 2. Summary of technologies employed to date for covalently attaching small molecules to solid supports in SMMs



- 1 Michael addition to maleimide-derivatised glass (SL Schreiber)
- 2 Activated (chlorinated) glass (SL Schreiber)
- 3 Staudinger ligation with phosphane-modified glass (R Breinbauer, CN Niemeyer and H Waldmann)
- 4 Hydrazide opening of epoxide-coated glass (I Shin)
- 5 Attachment to aldehyde-functionalised glass via an oxime or thiazolidine ring (KS Lam)
- 6 Attachment to gold-coated glass via a specialised SAM (M Frank)
- 7 Dipolar cycloadditions on non-covalently functionalised polystyrene (CH Wong)
- 8 Amide formation on NHS ester-functionalised glass (YT Chang)
- 9 Diels alder reaction with a dienophile-containing SAM on gold-plated silicon (M Mrksich)
- 10 Reaction of diazobenzylidene glass with heteroatoms bearing an acidic proton (SL Schreiber)
- 11 Immobilisation on to glass slides via carbene insertion (H Osada)

bound carbene intermediate that presumably reacts with the small molecule in an almost functional group-independent manner. This approach does away with the need to incorporate specific functional groups into the small molecule collection, rendering it useful for arraying the majority of commercial collections or sets of natural products.

One possible drawback of this technology is when the array is probed as the lowered concentration of molecules presented

The desire to array using functional group independent methods has also led researchers to explore noncovalent methods of localising small molecules on arrays (examples include the use of glycerol nanodroplets and biodegradable polymer spots). In summary, a good deal of creativity has been incorporated into methods of linking small molecules to glass slides (including the use of PNAs), but there remains no single answer to this complex problem.

Recent application of SMM technology

Having introduced the underlying chemical technology, the following briefly describes several success stories. The identification of a novel small molecule-binding partner for the yeast protein Ure2p has proved to be the most cited paper in this field to date.

The application of SMM technology in conjunction with fluorescently labelled recombinant protein enabled Schreiber and co-workers to carry out 3780 protein-binding assays in parallel. Uretupamine (1), the small molecule identified from the SMM, was shown to activate specifically a glucose-sensitive pathway downstream of Ure2p. An analogous approach was used by Schreiber to identify haptamide A (2), a small molecule that binds to and modulates the transcriptional activity of Hap3p, a subunit of the yeast Hap2/3/4/5p transcription factor complex. In this work, Schreiber clearly illustrates the importance of library synthesis in SMM research as the arrays used comprise some 12,396 small molecules prepared within his research group.

Chang, Yao and their co-workers have used SMMs to discover safe and cost-effective small molecule alternatives of Protein A and G for antibody purification and production. In this case, a triazine-based SMM was probed with fluorophore-conjugated human IgG. Recent successes have included identification of small molecules that bind to calmodulin and a move towards the integration of SMM with cell-based assays (Stockwell). In all of these cases, use of an SMM has led to the discovery of novel small molecules that will broaden our understanding of biological systems and/or will have a commercial impact.

In conclusion, this interesting technology links modern techniques in synthetic chemistry with micro-arraying techniques. Like all recently emerging research areas, it continues to adjust while the most robust and reliable combination of methods is identified. However, its potential to revolutionise the drug discovery process, chemical biology and beyond is clear. It can only be hoped that early predictions regarding the commercial availability of SMMs become a reality and that this technology is ultimately as accessible as its DNA/RNA parent. **END**