

Keeping ahead of the flow

Can the next generation of flow chemistry and biology platforms be fully integrated to transform the efficiency of small-molecule drug discovery for pharmaceutical industry?

There have been many false dawns in small-molecule drug discovery, promising individual technologies and integrated platforms to improve the efficiency of drug discovery. Although high-throughput screening, combinatorial chemistry and computational methods have all led to the rapid expansion of novel hit molecules and target-focused libraries, conventional medicinal chemistry is still the most effective way to optimise those early hits into the development compounds that can become drugs. This lengthy optimisation process is increasingly being outsourced to various locations worldwide with lower overheads and reduced long-term liabilities, as there has been no other expedient alternative to driving down costs to improve the earnings of pharmaceutical companies in the short term. However, effective integration and intelligent automation of the next generation of flow chemistry and biology technologies now has the real potential to transform this iterative process by enabling a step change in the efficiency, as well as a radical shortening of the timescales required to discover a candidate compound ready for development. It is envisaged by the authors that new small molecule integrated technology platforms will become sufficiently disruptive to challenge the efficiency of the latest monoclonal antibody discovery platforms.

Small-molecule drug discovery involves an iterative process of molecular design, chemical synthesis, biological assay and data analysis feeding directly into the next cycle. A typical medicinal chemistry project might encompass multiple cycles before a candidate compound is ready for development or the project is aborted. In particular, a complex range of assays are required to assess the potency and selectivity of a new molecule at the target of interest, as well as to assess *in vitro* the way the human body would deal with and respond to that molecule *in vivo* (eg metabolism and toxicity). Furthermore, the physical properties of the molecule, which will impact on the ease of

formulation of an eventual therapeutic, need to be taken into account. This iterative process takes time, involves the interaction of different multidisciplinary teams and it may be many weeks after the first synthesis of a molecule before a more complete picture of the biological and physical properties can be built, inevitably leading to the synthesis of molecules that retrospectively are of limited value. This process typically takes from a year to a decade, depending on the chemical starting point and degree of biological optimisation required, and involves at least one or more teams of skilled medicinal chemists.

The discovery process for small molecules

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Drug Discovery

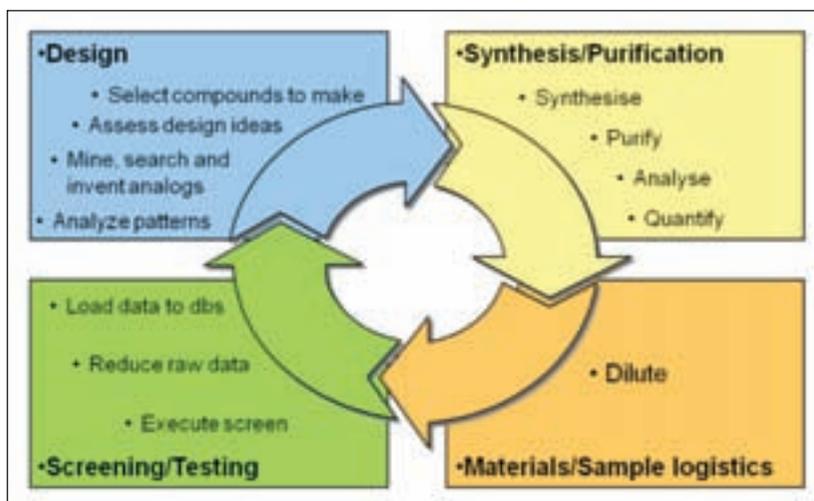


Figure 1

A fully-integrated fluidic workflow allows the seamless joining of synthesis through to biological assay alongside precise hardware control and integration of state of the art computational methods for compound design

described above contrasts with the equivalent process for the discovery of some biological therapeutics, during which a candidate product may be identified in a timescale of a few months, before more rapidly progressing into development. The discovery process for monoclonal antibodies (mAb) has been continually transformed and improved by new technologies over the past two decades. It is now possible to create a mAb specific to almost any extracellular or cell surface target and this technological revolution has driven a large amount of research and development with monoclonals for numerous serious diseases. Initially, murine antibodies were obtained by the now conventional hybridoma technology, which has proved very useful as a platform to generate pre-clinical research tools but allergic reactions in chronic use led to the overall clinical failure of most mouse antibodies, except in some specific circumstances. To reduce murine antibody immunogenicity, humanised antibodies are now produced by grafting murine amino acid domains into human antibodies, which results in a mAb of approximately 95% human sequence. As humanised antibodies often bind antigen more weakly than the parent murine monoclonal antibody, increases in antibody-antigen binding strength have been achieved by various specialised techniques including the introduction of mutation. New human monoclonal antibodies are now discovered using transgenic mice or phage display libraries. Human monoclonal antibodies are produced by transferring human immunoglobulin genes into the murine genome, after which the transgenic mouse is vaccinated against the desired antigen, leading to the production of monoclonal antibodies, allowing the transformation of murine

antibodies *in vitro* into fully human antibodies. In essence, the mouse does most of the required molecular optimisation of the mAb required for the biological target, with some reprogramming by modern molecular biology techniques¹.

For a small-molecule drug discovery platform to approach the level of efficiency of the above-mentioned mouse, a radically different approach to drug discovery needs to be adopted. The expeditious use of automation and emerging technologies now presents us with the opportunity to identify small molecules in a timeframe more usually associated with mAbs for the first time. This will lead to further downstream improvements to the overall efficiency of the pharmaceutical industry, as small molecule drugs have lower cost of goods and are, generally, easier to develop than mAbs.

A key requirement to enable both integration and automation is ensuring compatibility in process and hardware to realise the potential advantages. Identifying the most appropriate implementation of these individual processes to deliver the material and quality of data alongside the integration objectives is pivotal to the ultimate success of such a platform-based approach. It is now believed that methods in both chemistry and biology will facilitate this approach to yield a step change in the productivity in medicinal chemistry data generation².

The emergence of flow synthetic chemistry³ as a discovery research tool over recent years provides an alternate environment for small molecule synthesis with potential advantages for a range of chemical transformations. Indeed future opportunities include miniaturisation at the microfluidic scale which offers further potential while still providing more than adequate amounts of material for one or more *in vitro* bioassays.

Although typically undertaken in microtitre plate formats, a range of biological assays are now also feasible in a continuous flow format, indeed the concept of studying biochemical reactions in this flow format has been around for some considerable time⁴. Methods of detection demonstrated to date include mass spectrometry⁵, optical⁶ (eg fluorescence) and surface plasmon resonance. This approach will enable the integration of the biology with the chemistry, removing the somewhat onerous logistics requirements and allowing very rapid delivery of biological data for a newly-synthesised molecule, without the logistics overhead and having to resolubilise the test compound in aqueous buffers. This dramatic simplification, coupled with the potential advantages of speed accessible with an integrated and automated microfluidic

approach will enable the delivery of key structure activity relationship data in a timescale of hours rather than weeks or months.

Assays need not be limited just to biology, options exist to enable the determination of key physicochemical parameters, for example logD which will ensure molecules have not only the required biological profile but also measured properties to significantly increase their potential as future drugs.

Although the overall logic of the above arguments is hard to refute, a number of key challenges to achieve this level of integration and automation still remain. It is now believed that most technical hurdles can be resolved by the integration and adaption of existing technologies, in part through the exploitation of engineering and software expertise coupled with a pragmatic approach to technology R&D.

The flow paradigm now available for both synthesis and screening enables an overall platform approach whereby the small molecule is continuously processed through a series of connected fluidic processes. This requires a well thought through specification coupled with considerable knowledge and experience in the integration and engineering, particularly flow control of microfluidics, where the quality and control of the varied pumping requirements are key to success. While this may be trivialised to just some plumbing it remains without doubt a significant challenge for the integration.

Key to the generation of robust medicinal chemistry data is knowing that you have the right material, it is of high purity and known concentration. Fortunately modern HPLC systems now enable rapid purification and analysis with molecular structure confirmation by mass spectrometry. The amount of material is more challenging as simple UV methods are not quantitative, however, evaporative light scattering detection does now provide good levels of quantification provided the appropriate calibration methods are utilised.

An interesting difference between the chemical synthesis world and that of biological assay is the concentrations typically used. Flow synthesis, where reagents are typically at concentrations of low millimolar is still considerably in excess of those required in a typical biological assay. A high dynamic range dilution technique is consequently essential to make this connection. While simple methods can enable dilution ratios of 200-fold, an innovative approach was required to achieve the three orders of magnitude likely to be required to enable the full integration. Although a technical

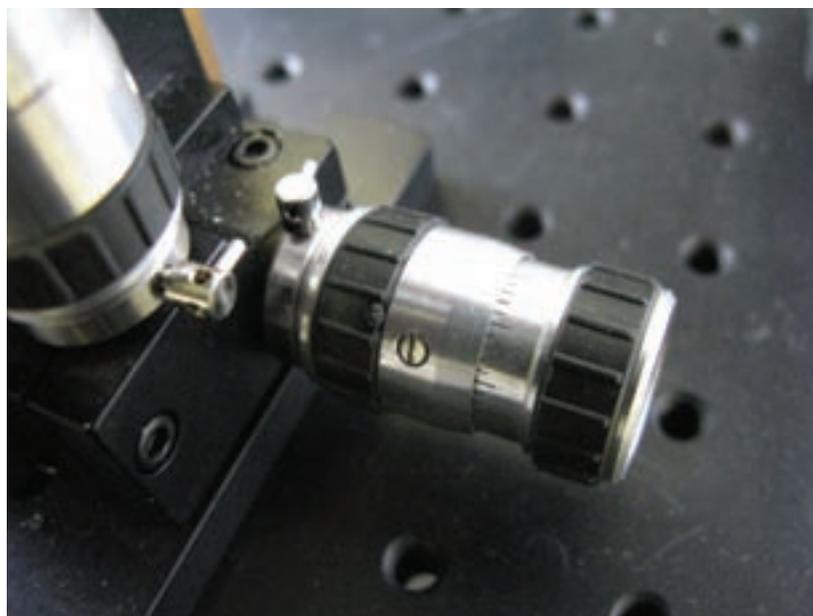
challenge, this approach does offer the advantage of diluting down any organics post-purification that may interfere with the assay. Indeed even the 200-fold dilution would mean a maximum organic concentration of 0.5% and in practice could be considerably lower than this.

Control of a range of different hardware components, even within a well defined process, is not a trivial exercise. The various components require integration and ideally should avoid the rewriting of existing component control software. A number of commercial solutions exist for the integration of laboratory hardware which enable the integration approach where the focus can be maintained on platform control and scheduling rather than the recreation of existing control software. Instrument providers can facilitate this through the provision of simple options for external hardware control and the ability to readily access data generated.

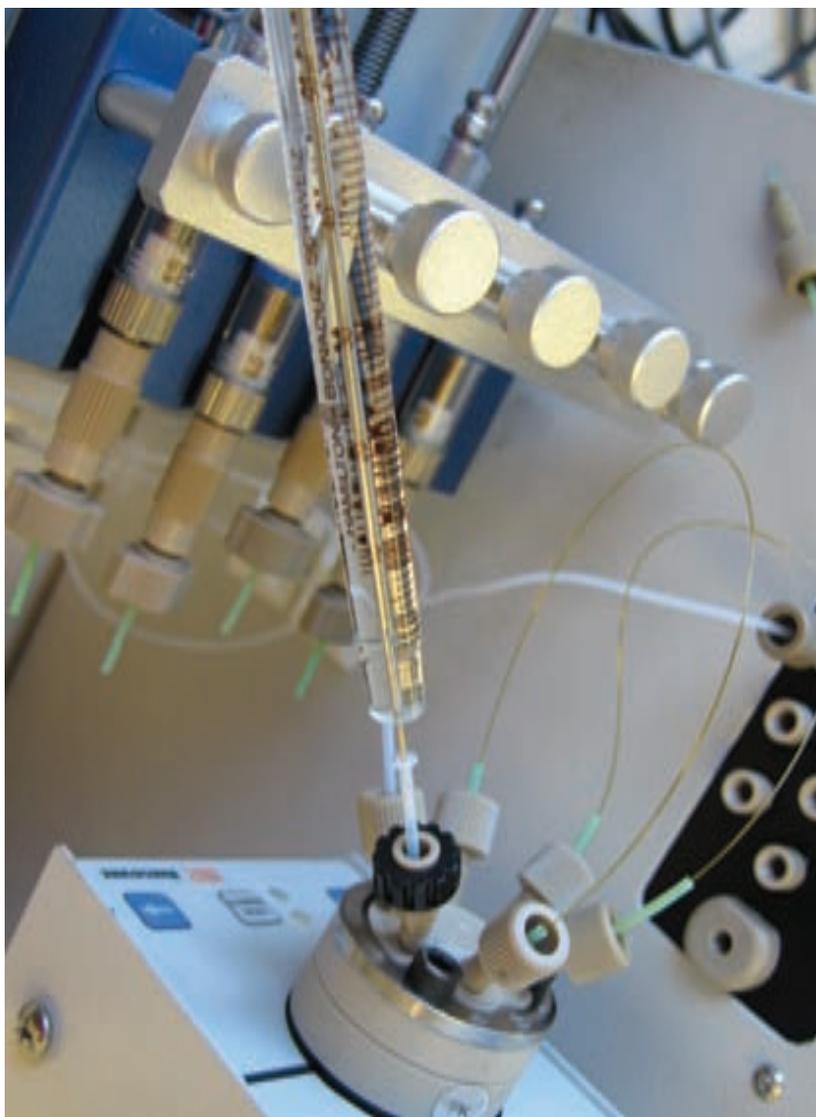
An integrated platform generates a wide range of data, both the experimental parameters for the molecules prepared (the laboratory notebook element) alongside the chemical analysis and biological assay data. There is a clear requirement for an integral informatics database that can readily capture, store and retrieve all the data associated with a particular experiment. This then provides the all-important structure activity data alongside the experimental methods to enable reproducing the results, both key to ensuring that the data will support intellectual property prevention.

Computational methods for the selection of molecules for drug discovery are constantly evolving and methods are now available that can assist the

Precision adjustment is required for alignment when working on the microfluidic scale



Drug Discovery



Accurate control of pumping and switching is key to working with low flow rates in microfluidics

medicinal chemist in proposing compounds for synthesis. These methods may be based upon simple calculated properties, for example the so called 'Rule of 5'⁸ to ensure experiments stay within relevant areas of chemical space as well as existing structure activity relationships and knowledge of the biological target. There are now a number of software tools which enable the sequential manipulation of data^{9,10}, the so called data pipelining approach (which the authors particularly like due to the similarity of the data manipulation approach to the continuous pipeline which facilitates the experimental data generation in the first place).

Will such a platform be able to identify candidate molecules just through loading up of reagents and initiating the process? The short answer is no, the philosophy behind the integrated make and screen paradigm is enabling the generation of data

to support the medicinal chemist in the identification of new drug molecules. The delivery of SAR data to the medicinal chemist, on a regular and frequent basis will enable such individuals to focus much more on molecular design, the key area where both a wide range of project experience combine with innovation to influence the design process and more rapidly deliver candidate quality molecules.

The fast iteration also provides opportunities for the identification of potent tool molecules to probe target tractability and mechanism of action. This offers significant scope for impacting on early stage discovery biology where research efforts may be hampered by inadequate tools to elucidate the biology.

Following up on hit finding campaigns will take on a new dimension. Currently, any one of a number of hit identification methods may identify a good number of interesting looking molecules which then go through a rigorous paper-based triage process to ensure only the most tractable are actually resourced in the laboratory. Given the high overheads of generation of SAR, even in the current paradigm of outsourced synthesis, this makes perfect sense, however once you have the ability to rapidly explore initial SAR in a matter of days or just a few weeks this becomes less sensible. Evaluation of a wider range of chemical hits, coupled with stringent design criteria, will enable a more thorough evaluation of the accessible chemical space which would be expected to impact positively on the final quality of candidates being produced.

A key element of the fully integrated approach is the rapid cycle time. Medicinal chemistry is the iterative generation of structure activity relationships and one might expect that the ultimate quality of molecules generated would, in part, be a function of the number of iterations. Beyond a certain point the speed of iteration will be dictated by the biological assay, particularly in advanced lead optimisation where the biological assay may include complex cell-based and *in vivo* data generation which inherently take long periods of time to complete. The generation of better quality molecules ahead of this time, through an increased number of iterations (in a significantly shorter period of time) will impact positively on quality.

What length of cycle time might ultimately be achieved? At present the integration of a straightforward two-step chemical synthesis with a simple biochemical assay would be expected within a two-hour window. This translates to up to 12 cycles in a 24-hour period. Looking just a little further forward, improved optimisation of chemistry and

biology may enable one-hour iterations, or up to 168 iterations in a week. Given a current estimate of at least one week per iteration in a more traditional pharmaceutical discovery environment, this clearly represents a paradigm shift in the early stages of small molecule discovery from initial hits to late stage molecules for final optimisation.

Consequently, integrated platforms will soon be capable of the very rapid generation of a wide range of chemical structures and corresponding biological activity data to potentially transform modern drug discovery. The acid test will now be the demonstration that a development candidate can be discovered in a dramatically shortened timescale. **DDW**

Dr J Mark Treherne has been actively involved in the biopharmaceutical industry for 25 years and was a co-founder and Chief Executive of Cambridge Drug Discovery Limited (CDD), leading the acquisition of CDD for £28 million by AiM-listed BioFocus plc, where he then became a Commercial Director of BioFocus, driving significant growth of its profitable services business. Since leaving his full-time position at BioFocus plc in February 2002 to remain on the Board as a non-executive director, Mark has now served on the Boards of more than 10 private and public biopharmaceutical companies. In addition to Cyclofluidic, he is currently on the Boards of NeuroDiscovery Limited, Domain Pharmaceuticals SA, Population Genetics Technologies Limited, Senexis Limited and Xention Limited. Dr Treherne has now helped raise more than £90 million for various biopharmaceutical businesses over the past 10 years. Dr Treherne was also formerly Chairman of ERBI Limited, which is a not-for-profit organisation that represents the biotechnology companies based around Cambridge in the East of England, UK. Dr Treherne initially trained as a neuroscientist and electrophysiologist at Cambridge University and previously led the Neurodegeneration research group at Pfizer's research facility.

Dr Dave M Parry has more than 17 years' experience in pharmaceutical discovery gained within the biotech and biopharmaceutical industry.

Following a BSc in chemistry from the University of Durham and a PhD in organic synthesis from Exeter, Dave spent two years in the USA working in the labs of Prof Philip D Magnus FRS. Starting his career as a medicinal chemist with Celltech, Dave rapidly developed an interest in drug discovery technologies and made the move to Chiroscience in Cambridge to head up its combinatorial chemistry effort. After the acquisition of Chiroscience by Celltech he continued to strengthen both his scientific and management skills leading the Exploratory Chemistry section at the Cambridge site, responsibilities including high throughput chemistry, analytical chemistry and the computational chemistry group. When UCB acquired Celltech in 2004, Dave had the opportunity to play a key role in small molecule discovery technologies and joined Cyclofluidic as Chief Operating Officer from UCB where he was most recently Director, Technology within Research.

Prior to joining Cyclofluidic as Chief Technology Officer, Chris Selway was employed at Pfizer Global R&D in Sandwich, UK as a Senior Principal Scientist. He brings considerable experience in pharmaceutical discovery technologies development and had been leading the development of a microfluidic synthesis-assay platform for hit-to-lead discovery within Pfizer. Following graduation with a BSc in Chemistry from Loughborough University, Chris has accumulated 26 years of experience in pharmaceutical research at Pfizer, and has broad knowledge and expertise in chemical technology development and modern organic synthesis techniques. He was a founder member of the Pfizer Library Design and Production group with responsibility for development and implementation of automated systems for synthesis, analysis and purification of compound libraries. In 1997 Chris was awarded a Pfizer Individual Achievement Award for outstanding contribution to parallel synthesis and chemistry automation and in 2000 was awarded a Pfizer Team Achievement Award as member of the Autopurification Team for innovative design and delivery of a world leading system for the efficient purification of library compounds. More recently Chris has focused on applying flow chemistry and microfluidic technologies to enhance the drug discovery process. Externally, Chris is currently collaborating with Professor Steve Ley at Cambridge University, with a focus on flow chemistry; and is a member of the organising committee of the United Kingdom Automated Synthesis Forum (UKASF).

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- 9 For example Labview from National Instruments.
- 10 For example Knime (www.knime.org) and Pipeline Pilot (www.accelrys.com/products/pipeline-pilot).