The key to the approach was to 'disguise' a reactant's hydroxyl group by converting it into a silyl ether — a silicon-containing group that is known to bind to metal catalysts (Fig. 1). The silyl ether serves as a directing group by forming a strong attachment to the iridium catalyst and directing the formation of a carbon–silicon bond at a specific C–H bond elsewhere in the reactant. In a subsequent step carried out in the same reaction vessel, Simmons and Hartwig removed the silyl ether mask by oxidizing the carbon–silicon bond to a C–OH bond, liberating the desired 1,3-diol product.

The authors demonstrated the power and utility of their method by converting a variety of simple and complex alcohols to 1,3-diols. In every case, the functionalization occurred at a single C–H site three carbon atoms away from the directing group, even if more-reactive C–H bonds were present elsewhere in the molecule. Impressively, the site selectivity remained high even when Simmons and Hartwig subjected structurally complex natural products to the reactions. They were therefore able to convert readily available natural products bearing a hydroxyl group into other natural products that are more difficult to obtain, and to generate new analogues of natural products.

A particularly striking example is the authors' synthesis of methyl hederagenate (see Fig. 3b of the paper[1]) — a precursor of the natural product hederagenin, which has anti-inflammatory, antifungal and antitumour properties[9]. Although the starting material for the synthesis (commercially available methyl oleanate) contains 49 C–H bonds, only one of these bonds was selectively functionalized by their three-step reaction. Previously, the most efficient synthesis[10] of hederagenin required ten steps from a starting material closely related to methyl oleanate.

Although Simmons and Hartwig's method is very useful for the functionalization of substrates that contain only one hydroxyl group, it would have even greater application if it could be used for molecules that have several hydroxyl groups. Whether selectivity can be obtained in such systems remains to be seen. Nonetheless, the authors' innovative transformation sheds light on how common chemical groups can be used to direct C–H functionalization reactions, and provides a new and efficient way to prepare 1,3-diol units in complex organic molecules. ■

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MICROFLUIDICS

Analog-to-digital drug screening

Current methods for screening libraries of compounds for biological activity are rather cumbersome, slow and imprecise. A method that breaks up a continuous flow of a compound's solution into droplets offers radical improvements.

ROBERT C. R. WOOTTON & ANDREW J. DEMELLO

Finding new leads for drug discovery increasingly depends on high-throughput methods that allow efficient biological screening of chemical libraries. Reporting in *Proceedings of the National Academy of Sciences*, Miller *et al.*[1] describe a method for screening large numbers of compounds — thousands or more — for their activity as enzyme inhibitors. This protocol generates immense amounts of high-precision dose–response data in ultrashort times of just a few minutes per compound. The authors' approach could vastly improve the efficiency of current screening processes, which represent a major bottleneck for drug-discovery programmes.

Conventional routes to screening compounds typically begin with a qualitative primary screen of a diverse compound library to identify which compounds are active at a biological target. This is supplemented by a dose–response analysis of selected active compounds, to relate the target's activity to each compound's concentration. Unfortunately, dose–response screening of libraries is a time-consuming and complex procedure, with many potential pitfalls. Not least of these is the amount of time required to gather sufficient data to probe complex pharmacology, and the large number of false positives and false negatives generated from standard assays[2].

Library screening to find enzyme inhibitors in particular can be fraught with difficulties because of the high number of compounds that must be screened and the rather cumbersome methods used — typically involving robots running assays in tiny wells on plates. In practice, every compound in a primary screen is normally assayed at a single concentration; the vast majority of candidates are almost inevitably inactive and are thus eliminated. The first screen is then followed by a more complex, dose-dependent assay of the remaining candidates. But although secondary assays are more detailed, they are frequently run at only a limited number of compound concentrations, because they face similar constraints to the primary screen[3].

Assays based on microfluidics — the precise control of fluids constrained within sub-millimetre-scale channels — could transform high-throughput screening. Microfluidic technology is being increasingly used in chemistry and biology because of its ability to rapidly perform complex analytical procedures on minute sample volumes, with greater efficiency than traditional macroscale approaches[4]. Such systems normally operate either by passing a continuous flow of a solution through channels, or by breaking a flow into segments or droplets.

Continuous-flow systems offer some advantages over those involving segmented flow — for example, they allow chemical gradients to be established in a flow. But they are also hampered by sample dispersion, which spreads a compound sample out from an initial, focused volume to a wider, more dilute region. Continuous-flow systems also suffer from residence-time distributions, in which some parts of a sample move faster than others, thus adding to sample spreading and dilution. Segmented (or droplet-based) flows, on the other hand, offer excellent control over sample dispersion and residence time, although gradients are more difficult to establish.

Miller *et al.*[1] have exploited key features of both continuous- and segmented-flow microfluidics to develop a robust system capable of generating high-quality dose–response data in screens for enzyme inhibitors. At the heart of their approach is the generation of a concentration gradient over time in a flow of a solution of the compound using Taylor–Aris dispersion (Fig. 1a) — a phenomenon that occurs in continuous flows as a result of a

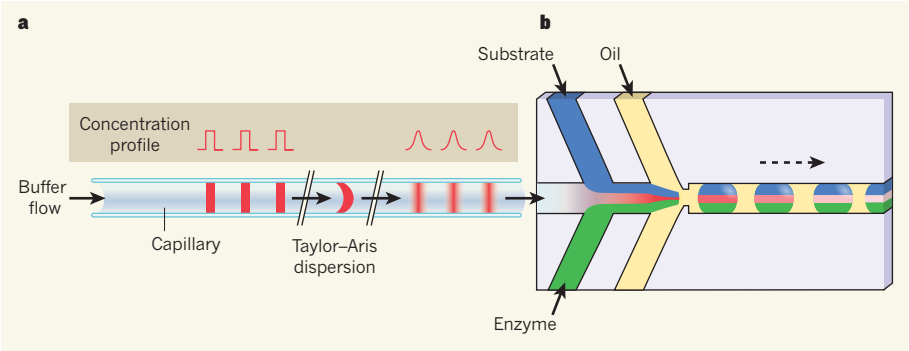

Figure 1 | High-resolution dose–response screening. Miller *et al.*[1] report a microfluidics system that enables the activity of enzyme inhibitors to be measured at thousands of different concentrations in just a few minutes. **a**, In their system, solutions of inhibitors (red) are injected into a continuous flow of buffer in a capillary tube. By means of a phenomenon known as Taylor–Aris dispersion, the concentration profile of each injected solution is transformed into a smooth pulse. **b**, Solutions of the targeted enzyme and of an enzyme substrate are introduced into the flow, which is then broken into droplets by the addition of an immiscible oil. Each droplet thus contains a slightly different concentration of the inhibitor. By measuring enzyme inhibition within each droplet, a dose–response curve can be plotted for the inhibitor. (Figure adapted from ref. 1.)

combination of molecular diffusion and the variation of flow velocity over the cross-section of a microchannel. The authors segment this flow into a series of droplets dispersed in a continuous flow of oil (Fig. 1b), in a process similar to the analog-to-digital conversion of an electrical signal.

Each droplet has a volume of just 140 picolitres (1 picolitre is 10^{-12} litres), and defines a unique reactor containing a concentration of the candidate inhibitor that differs from the concentrations in droplets preceding or following it. No dispersion of the dissolved compound can occur between droplets, and so the concentration gradient established in the original continuous flow is preserved. By incorporating the target enzyme and its substrate within each droplet and measuring the enzyme inhibition by the candidate, the authors were able to produce dose–response curves containing 10,000 data points for a compound from a single sample of solution, within a few minutes.

The large amount of data extracted from each experiment means that the resulting dose–response curves can be analysed with high precision using complex kinetic models. This greatly reduces the occurrence of false negatives and false positives, and generates much more helpful data than are produced by currently available secondary assays. Given that the authors' microfluidic device can screen each compound so effectively within two to three minutes, their approach also opens the way to more thorough and effective primary screening.

As Miller *et al.* highlight[1], their system still has some issues that must be resolved, such as ensuring that each droplet remains in the microfluidic system long enough for enzyme inhibition to occur. The authors currently achieve this by incorporating channels (known as delay lines) in which the flow is slowed. Although these lines allow reactions to be assayed over a period of 3.5 minutes, they also introduce residence-time distributions because of droplet disordering. This is clearly problematic, especially when screening slower enzymatic processes, but could be overcome by changing delay-line geometry and channel lengths. Another concern is that the range of inhibitor concentrations that can be accessed by the authors' system is rather limited. However, this issue could easily be resolved by incorporating passive droplet-dilution modules[5] — devices that would dilute each sample-containing droplet into a stream of new, more-dilute droplets.

Despite these limitations, the impact of Miller and colleagues' approach on the drug-discovery process will be considerable. By enabling large amounts of dose–response information to be obtained from small sample volumes, it could facilitate the direct, quantitative dose–response screening of whole libraries in short times, eliminating the need for second-pass screening of active compounds. ■

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TRANSLATIONAL MEDICINE

Primed for resistance

A drug for treating melanoma is ineffective in colorectal cancers that have the same causative mutation. Studies of how cells adapt to the drug reveal why this is so, and suggest combination therapies that may be more effective. SEE LETTER P.100

DAVID B. SOLIT & PASI A. JÄNNE

Genetic mutations that disrupt fundamental cellular processes, such as cell division and differentiation, can lead to cancer. Mutations in the *BRAF* gene, which codes for the kinase protein BRAF, are one such example, and are detected in multiple cancers. In patients with advanced *BRAF*-mutant melanomas, treatment with the BRAF-inhibiting drug vemurafenib significantly prolongs survival[1]. However, vemurafenib is ineffective[2] in patients with colorectal cancers that harbour the same *BRAF* mutation — *BRAF*(*V600E*). On page 100 of this issue, Prahallad *et al.*[3] provide an explanation for this lack of efficacy by showing that, in colorectal cancer cells, drug treatment leads to rapid activation of a protein that counteracts the drug's BRAF-inhibitory activity.

Prahallad and colleagues identified this molecular basis for resistance by screening for genes for which inhibition of expression leads to enhanced sensitivity of human colorectal cancer cells to vemurafenib. Their screen assessed all 518 human kinase-encoding genes and 17 additional kinase-related genes. Among the most potent 'hits' they identified was the gene coding for the epidermal growth factor receptor (*EGFR*). To test the biological significance of this finding, the authors treated *BRAF*(*V600E*)-mutant colorectal cancer cell lines with vemurafenib, and found that the drug induced hallmarks of EGFR activation.

EGFR acts earlier than BRAF, and other RAF proteins, in a cell signalling pathway called the ERK pathway. The authors hypothesize that inhibition of BRAF by vemurafenib relieves a negative feedback loop that keeps EGFR inactive. EGFR activates a protein called RAS, which, when activated, has been shown to cause vemurafenib resistance by inducing the formation of dimers of RAF proteins, against which the drug is ineffective (Fig. 1). EGFR also activates other cell signalling pathways,