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Control and detection of chemical reactions in microfluidic systems

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Recent years have seen considerable progress in the development of microfabricated systems for use in the chemical and biological sciences. Much development has been driven by a need to perform rapid measurements on small sample volumes. However, at a more primary level, interest in miniaturized analytical systems has been stimulated by the fact that physical processes can be more easily controlled and harnessed when instrumental dimensions are reduced to the micrometre scale. Such systems define new operational paradigms and provide predictions about how molecular synthesis might be revolutionized in the fields of high-throughput synthesis and chemical production.

Ever since Wöhler's laboratory synthesis of urea in 1828 (ref. 1), the chemist's toolkit has predominantly consisted of macroscopic components fabricated from glass. Examples include round-bottomed flasks, test tubes, distillation columns, reflux condensers and retorts. Despite advances in experimental and mechanistic organic chemistry during the past century, it is noteworthy that the basic experimental techniques and associated equipment have remained largely unchanged. There are a number of reasons why traditional synthetic chemistry is performed in the aforementioned equipment, but is there any advantage to performing synthetic chemistry in volumes 5–9 orders of magnitude smaller than those associated with bench-top chemistry? As shown below, the application of techniques cultivated in semiconductor industries have allowed the creation of a new instrumental platform able to efficiently manipulate, process and analyse molecular reactions on the micrometre to nanometre scale. Even at this early stage in the development of 'microfluidic' reaction systems, it is clear that advantages engendered by miniaturization may affect molecular synthesis similarly to the way that the integrated circuit has defined the computer revolution over the past 50 years.

Flow and mixing on the microscale

A primary reason why microfluidic systems provide unusual environments in which to perform synthesis is the dependency of fluid-flow characteristics on scale. Although many diverse effects manifest themselves upon moving from macroscale to microscale environments, some critical features are worthy of discussion.

A tangible effect of reactor miniaturization is that fluid properties become increasingly controlled by viscous forces rather than inertial forces (see page 374). For microfluidic systems, such as blood capillaries, Reynolds numbers (Re) are typically $<10^2$. This represents a situation in which flow is considered essentially laminar, and contrasts with macroscale conduits ($Re>10^3$) in which flow regimes are almost always turbulent. This behaviour has a direct consequence on mixing within microfluidic systems. Before a reaction between two reagents can occur, intimate contact between the component molecules must be realized through mixing. In its simplest manifestation, this occurs by uniting pure fluid-component streams. Because mixing can only be accomplished by diffusion, rather than through the fast convective processes that dominate in turbulent systems, the only route to mixing is diffusion across fluidic interfaces (Fig. 1a). Diffusive mixing efficiencies for continuous-flow systems can be measured using the Fourier number, and indicate that mixing timescales increase with the characteristic dimensions of the reactor. Consequently, although mixing via diffusion is inefficient for reactors with characteristic dimensions greater than 1 mm, when diffusion distances drop below 100 μm mixing times can, in theory, become very small.

The ability to controllably and rapidly create a homogenous reactant mixture at the commencement of a reaction is desirable. Indeed, the effect of mixing on the extent of a reaction and product distribution is crucial in reactor design. It is generally recognized that first-order irreversible reactions are not affected by local turbulent mixing, but by the residence time of the system, and conversions can therefore be easily calculated[2]. However, in the case of fast reactions in which two or more reagents are initially present in separate streams, reaction rarely occurs uniformly throughout the whole volume. The rate of reaction is no longer defined by inherent kinetics, but is limited by diffusional rates. Thus, for fast reactions yielding a single product, yield is regarded as a direct measure of the degree of mixing.

The relationship between the reaction rate and the rate of mixing can be reduced to one of three general categories; the chemical regime, the diffusional regime and the mixed chemical/diffusional regime. In the chemical regime, mixing is fast compared with the reaction rate (and is complete before a significant amount of product is generated). In the diffusional regime, reaction is fast, with the rate being limited by the mixing speed. In this case, the reaction rate is independent of the rate constant, and the formation of secondary products in this situation is greatest. Finally, in a mixed chemical/diffusional regime the greatest interaction between chemical reactions and fluid dynamics occurs, and the product distribution depends on both chemical factors (such as reaction kinetics) and diffusional factors (such as the mixing efficiency). To address these variations, a diverse range of microfluidic systems have been designed for the rapid mixing of fluids[3,4]. They can all be broadly classified as being either passive or active. Passive mixers rely on geometric properties of the channel or fluidic streams to maximize the area over which diffusion can occur, whereas active mixers rely on time-dependent perturbations of the fluid flow to achieve mixing. However, the fact that spatial organization of fluid streams allows mixing to be performed in an extremely rapid and controllable fashion is

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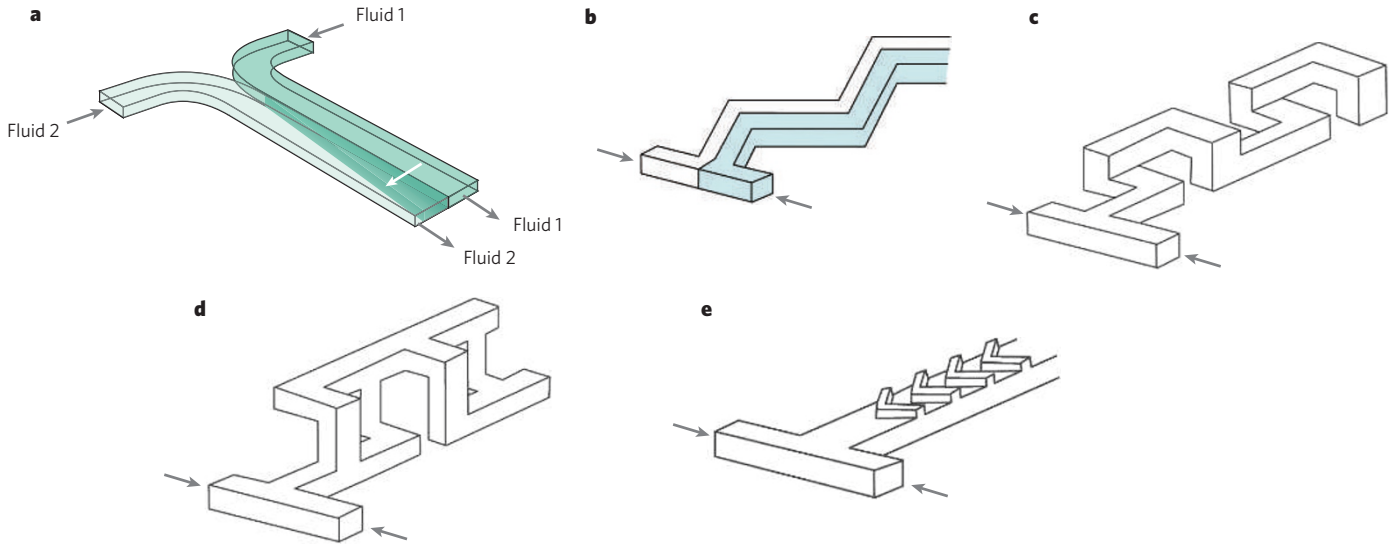

Figure 1 | Microfluidic approaches for mixing in continuous flow. **a**, Mixing of two miscible fluid streams under laminar flow conditions. The component streams mix only by diffusion, creating a dynamic diffusive interface with predictable geometry. **b**, Zigzag-shaped channel for chaotic mixing at high Reynolds numbers[9]. **c**, Three-dimensional L-shaped channel for chaotic mixing at intermediate Reynold numbers[11]. **d**, Three-dimensional, connected out-of-plane channel for chaotic mixing at intermediate Reynold numbers. **e**, Staggered-herringbone grooves for chaotic mixing at low Reynolds numbers[12].

common to all approaches. Both features have significant advantages over macroscale systems and offer potential solutions to a number of key problems faced in contemporary synthesis. These include the ability to probe ultra-fast chemical reactions (with minimal sample consumption), which is beyond the reach of current technologies[5]. An excellent example of such facility was described by Knight *et al.*, who reported a continuous-flow mixer incorporating a hydrodynamic focusing geometry with mixing times of less than 10 μs and sample consumption rates of nanolitres per second[6].

Passive mixers have found the widest use in synthetic applications due to their simplicity and operational flexibility. Although operation within laminar-flow regimes can provide rapid mixing if diffusional distances are kept small, in many situations practical limitations (such as minimum feature dimensions) mean that basic flow lamination is inefficient at generating high degrees of mixing within short times. However, rapid mixing with low reagent consumption is achievable using chaotic advection. Put simply, chaotic advection enhances mixing in laminar-flow systems, because it acts to continuously 'stretch' and 'refold' concentrated solute volumes, thereby creating an exponential decrease in striation thickness[7,8]. Chaotic advection in microfluidic systems can be achieved by introducing obstacles within channels or on channel surfaces, or by modifying channel geometries. In each case, the modification acts to enhance stretching, folding and breaking of the flow. For example, zigzag channels (Fig. 1b) can generate chaotic advection at high Reynolds numbers by recirculation around turns[9], whereas three-dimensional serpentine channels (Fig. 1c, d) consisting of repeating segments in orthogonal planes can generate chaotic flows at low to intermediate Reynolds numbers[10,11]. Creation of chaotic flow at low Reynolds numbers has also been established through the use of grooves on channel surfaces (Fig. 1e). A good example of this approach was reported by Strook *et al.*, who used bas-relief, herringbone grooves on a channel bed to induce chaotic mixing at Reynolds numbers between 1 and 100 (ref. 12). More recent studies have also used surface-charge patterning to create electrokinetic mixing in low Reynolds number regimes[13].

A significant problem encountered in single-phase microfluidic systems is that of achieving rapid and efficient mixing of fluids while minimizing dispersion. Under most circumstances, channel walls impart shear forces on the contained fluid, so under applied hydrodynamic pressure a parabolic velocity profile is established over the cross-section with fluid velocity zero at channel walls and maximum at the centre. The chief implication of this behaviour is that a reaction mixture sampled after initiation of mixing is formed from an ensemble of volume elements that have spent varying times on-chip. This yields a residence-time distribution that may cause significant variation in the yield, efficiency and product distribution of a reaction. Localization of reagents within discrete droplets is an effective way of eliminating this phenomenon. Several recent studies have exploited the formation of droplets in microfluidic systems to perform a variety of analytical processes[14–16]. Of particular note are those that use flow instabilities between two immiscible fluids[17]. As can been seen in Fig. 2, droplets can be made to form spontaneously when multiple laminar streams of aqueous reagents are injected into an immiscible carrier fluid[18]. The formed droplets define picolitre volumes, and because each droplet is isolated from channel surfaces and other droplets, each one acts as an individual reaction vessel. Variation of the cross-sectional dimensions of microchannels can be used to regulate droplet volumes, and flow-rate variation allows control of reagent concentrations[16]. Importantly, the use of twisting channel geometries is effective in generating chaotic mixing within droplets, by folding, stretching and reorienting fluid. Consequently, mixing is rapid and reagent transport occurs with no dispersion. Such features, combined with the ability to combine, split and sort droplets, are likely to transform the application of microfluidic systems, and suggest that they would be of use in high-throughput synthesis (due to high sample throughput) and kinetic measurements (due to low sample requirements and negligible dispersion)[19].

Synthetic unit operations

High surface-to-volume ratios are key in defining fluid-flow characteristics at the microscale. Of equal importance is the effect of these ratios on diffusion-mediated mass and heat transfer in reactive processes. For example, typical microfluidic devices exhibit high thermal-transfer efficiencies by virtue of reduced thermal masses and high surface-to-volume ratios, and therefore allow exothermic and/or high temperature reactions to be performed in an efficient and controllable (isothermal) manner. Microfluidic systems have been created to allow efficient biphasic reactions between elemental fluorine and a range of organic substrates. Because the transformation of a carbon–hydrogen bond to a carbon–fluorine bond using fluorine is highly exothermic ($\Delta H = -430$ kJ mol^{-1}), safety issues relating to temperature control are of vital importance, especially on a large scale. Indeed, studies by Chambers *et al.* have reported direct fluorination of a range of substrates, including diketones and ketoesters[20–22]. Other examples

in which microfluidic environments have been shown to provide for efficient temperature and thus reaction control include continuous-flow reactors for multicomponent reactions[23], Swern oxidations[24], diazotizations[25,26], nitrations[27], Andrussow reactions, Reimer–Tiemann formylations[28] and carbonylations[29]. A wide variety of other reactivities have been demonstrated in microfluidic reactor systems, including catalytic hydrogenations and dehydrogenations[30], Suzuki couplings[31], Grubbs metathesis[32] and photochemical reactions[33,34]. As these and many others have been discussed elsewhere[35–39], only a small number of recent and illustrative examples are described herein.

In recent years, developments in genomics and proteomics have generated many potential drug targets, each requiring small-molecule modulators. These demands have prescribed massive investment into synthetic technologies that can produce drug candidates on short timescales. The vast majority have used solid-supported chemistry to generate compound libraries.

Unfortunately, the dependence on solid-support technologies has severely limited our ability to perform high-throughput molecule discovery[40]. Put simply, efficient attachment and detachment to and from the support are crucial for successful library generation, increasing the number, time, cost and complexity of process steps and the amounts of required reagents. Moreover, reaction rates of solid-phase reactions are appreciably slower than the corresponding solution-phase processes. Accordingly, the reasons to pursue solution-phase chemistries for library generation are undeniable. The batch nature of conventional solution-phase methods (which use arrays of microwells) is unsuitable for efficient process optimization and high-throughput processing. Conversely, the control of fluidic reagent streams within microfluidic systems allows reactions to be performed within chemical regimes (in which mixing is rapid and reaction timescales are defined by inherent reaction kinetics), whereas operation in a continuous- or segmented-flow format allows compartmentalization and/or spatial identification of multiple reactions[41].

An early demonstration of the use of microfluidics in small-molecule compound-library generation was reported by Mitchell *et al.*, who used distributive mixing of laminar reagent streams to synthesise α-dialkylacetamide libraries[23,42]. Reactions were performed in a serial (time-encoded) or parallel (mass-encoded) fashion, and real-time product identification and quantitation was achieved through integration of the microfluidic reactor with time-of-flight mass spectrometry. Such a combination afforded unprecedented control over the reaction, and real-time identification of the small-molecule products. Further utility of microfluidic systems in making compound libraries has been shown by Garcia-Egido *et al.*, who prepared a series of 2-aminothiazoles by means of a Hantzsch reaction of ring-substituted 2-bromoacetophenones and 1-substituted-2-thioureas[43]. Additionally, Fernandez-Suarez *et al.* reported automated sequential solution-phase combinatorial synthesis to perform a 2×2 synthesis using the Knoevenagel condensation of 1,3-diketones and aldehydes[44]. The system was configured so as to allow multiple reagent streams to be introduced sequentially under hydrodynamic flow. Subsequent development of this concept allowed the rapid and automated synthesis and analysis of a 7×3 pyrazole library[45].

In reality, sequential systems are limited in terms of application to high-throughput synthesis. In addition to the possibility of cross-contamination, detection systems are limited to those that can effectively probe small volumes. Consequently, approaches to parallel, solution-phase synthesis in microfluidic reactors have been investigated. Parallel solution-phase synthesis is typically complex and inefficient, requiring multiple reactors and large reagent volumes. Accordingly, the development of microfluidic technologies to efficiently process small volumes of reagents within monolithic devices would represent a significant advance. A step towards integrated, parallel-reaction systems was presented by Kikutani *et al.*[46]. To prove principle, a 2×2 parallel-reaction scheme was transferred to a chip-based format. The primary concern when creating a monolithic system lies in the complex channel topology required to perform reactions in a parallel fashion. Most notably, for an

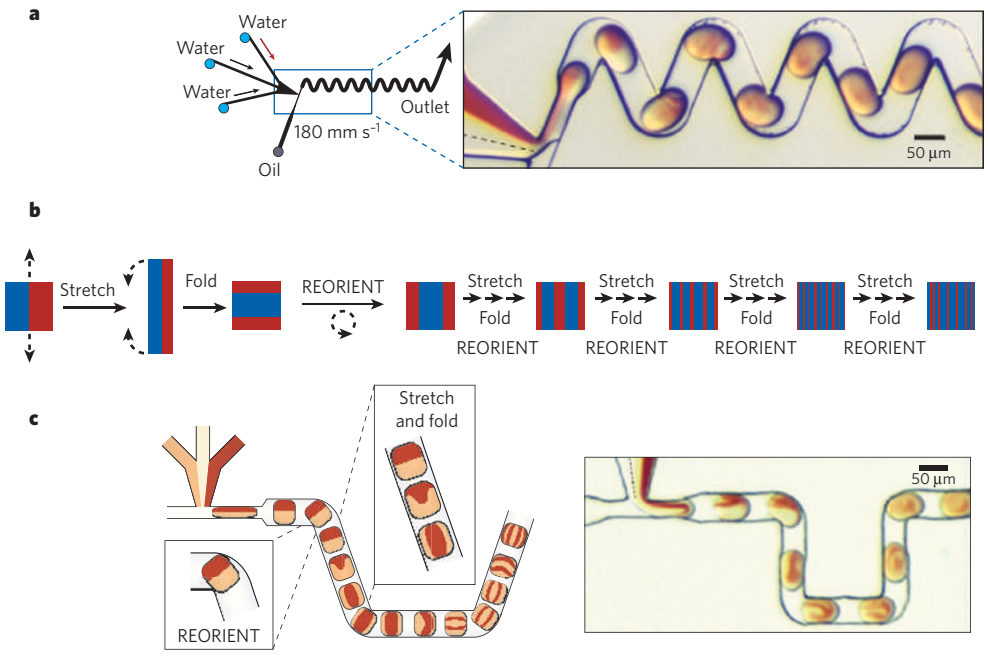

Figure 2 | Formation of microdroplets in microchannels. Microfluidic droplets can be made to spontaneously form when multiple laminar streams of aqueous reagents are injected into an immiscible carrier fluid. The formed droplets define picolitre volumes, and because each droplet is isolated from channel surfaces and other droplets, each one acts as an individual reaction vessel. Chaotic advection within droplets moving through winding channels is used to generate rapid mixing. **a**, Mixing in plugs. Arrow colour corresponds to dye in stream solution. Dashed line in image indicates merging of the streams. (Image reproduced, with permission, from ref. 16.) **b**, Diagram of a fluid element undergoing stretching, folding and reorientation (known as the baker's transformation). Repetition of this process leads to decrease of the striation thickness and facilitates efficient mixing. **c**, Microphotographs of a microfluidic network in which flow patterns inside plugs in the microchannel clearly demonstrate flow patterns reminiscent of the baker's transformation. Red aqueous streams are solutions of $[Fe(SCN)_x]^{(3-x)+}$ and colourless aqueous streams KNO_3 solution. The oil stream is a solution of water-immiscible fluorinated fluid (perfluorodecalin) with a 10/1 volume/volume ratio of 1H,1H,2H,2H-perfluoro-1-octanol. (Image reproduced, with permission, from ref. 16.)

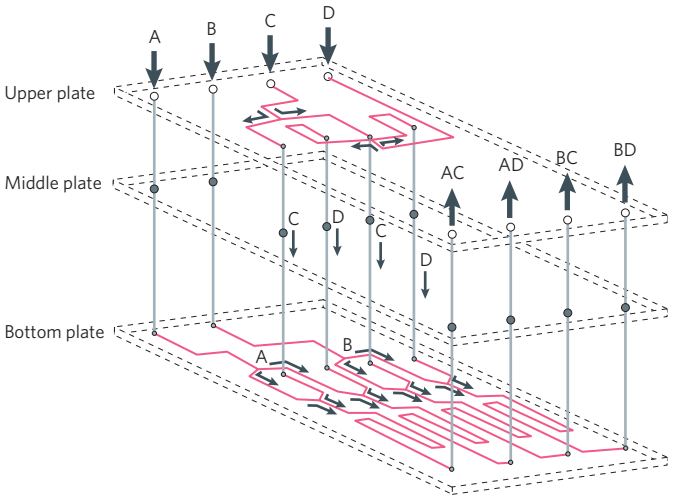

Figure 3 | Schematic view of three-dimensional microchannel circuit for performing parallel combinatorial chemistry. For parallel synthesis, reagents are distributed to each microchannel reactor in a defined manner. There are four inlets and four outlets. Reagent pairs (A and B, and C and D) are libraries of starting molecules. The flow of each reagent is divided into two streams and mixed in four different combinations: AC, AD, BC and BD. An equal distribution of reagents at the branching points is achieved by making the channel length after the branching points equal for both sides[46].

n by *m* (compound) combinatorial system in which *n* and *m* are greater than 2, a three-dimensional channel network is necessary. To achieve this, two glass substrates were lithographically structured to define two fluidic layers (Fig. 3). Using this approach, a 2×2 combinatorial amide formation reaction was performed with product yields in excess of 90%. Importantly, no impurities or cross-contamination were observed. Although elegant, it is doubtful whether such an approach will ever find application in the synthesis of large libraries due to an associated increase in the complexity of the required fluidic circuitry.

In addition to homogeneous reactions, the large surface-to-volume ratios characteristic of microfluidic reactors provide unique environments for performing heterogeneous chemistry. To this end, Kobayashi *et al.* recently reported enhanced efficiencies of gas-liquid-solid hydrogenation reactions in microchannels[47]. By encapsulating a palladium catalyst in a copolymer matrix attached to the microchannel surface, the metal remains active while irrevocably bound in the solid phase. Using this approach, various substrates (including double bonds, tri-substituted olefins, and triple bonds) were reduced using an annular-flow system. Reactions went to completion within 2 min, and space-time yields were five orders of magnitude higher than equivalent laboratory-scale reactions. The approach is also suitable for large-volume chemical synthesis via 'scale-out', and, importantly, opens up the opportunity for performing a range of catalytic processes at high speed and with negligible catalyst leaching.

Although the above studies provide persuasive arguments for using microfluidic systems in high-throughput synthesis, it is apparent that application is defined by the ability to develop both complex and efficient world-to-chip interfaces, which allow easy coupling between multiple reagent reservoirs and the microfluidic device[48]. At present, reports of microfluidic systems for combinatorial chemistry have, at best, proved principle. Nonetheless, these developments define new paradigms for high-throughput molecular synthesis and provide some of the most credible predictions about how the true power of combinatorial synthesis may be harnessed in molecular discovery.

Enabling nanomaterial synthesis

As I have highlighted, much of the interest in using microfluidic systems for synthetic applications lies in their ability to perform rapid and controllable mixing. This, combined with manipulation of variables such as temperature, concentration gradients and pressure, dictates that continuous-flow processing on the microscale can be used to synthesize species of specific yet variable characteristics. Perhaps the most interesting demonstration of this feature has been the use of microfluidic reactors to synthesize nanomaterials of defined size and anisotropy. Nanomaterials exhibit optical and electronic properties that depend on their size and shape, and are seen as tailored precursors for functional materials in biological sensing and optoelectronics[49]. These critical dependencies indicate that 'bottom-up' approaches for nanomaterial synthesis must provide for fine control of the physical dimensions of the final product. Synthetic routes involve the particle growth on an atom-by-atom basis, and have been used to create spherical, cubic, tubular and tetrahedral crystallites of well-defined size and shape[50]. Bottom-up approaches have attracted interest owing to their versatility and ease of use, but for many applications deviations about the mean particle diameter must be <1% to achieve the desired selectivity. This is beyond the tolerance of standard macroscale syntheses, and it is almost always necessary to use some form of post-treatment (including chromatography, sedimentation, precipitation and photocorrosion) to extract the desired particle size[51]. Accordingly, nanoparticles with narrow size distributions can be extracted, but because the starting point for all such methods is a polydisperse sample, product yields are low.

An ideal recipe for nanoparticle synthesis must ensure that nucleation of solute molecules (to form 'seed' particles) occurs on a timescale that is short compared with the characteristic growth time (in which the seeds capture dissolved solutes). Moreover, nucleation and growth should occur in an environment in which chemical state functions are precisely controlled[52]. If these conditions are not met, the size of critical nuclei and growth rates will vary according to location, and result in a distribution of particle sizes. In many respects, microfluidic systems provide an ideal medium for nanoparticle production. Because both mass and thermal transfer are rapid, temperatures may be defined with precision or varied on short timescales. Additionally, reagents can be rapidly and efficiently mixed to ensure homogeneous reaction environments, while allowing for additional reagents to be added at predefined times.

Recent studies have demonstrated that microfluidic reactors drastically outperform macroscale systems in the direct production of nanoparticles. Using simple flow regimes whereby component streams are mixed at low Reynolds numbers and in continuous flow, variations in reaction residence times, temperatures and reagent concentrations are used to control average particle size, while sample size distributions are minimized through a reduction in residence-time distributions and precise control of chemical state functions. In this way, high-quality cadmium sulphide[53,54], cadmium selenide[55,56], palladium[57], silver[58,59], gold[60], copper[61], titania[62] and CdSe–ZnS core–shell nanoparticles[63] have all been synthesized directly.

Recent studies have addressed the issue of further minimizing particle size distributions through the development of segmented-flow reactors. Shestopalov *et al.* demonstrated a two-step chemical synthesis of colloidal CdS and CdS–CdSe core–shell nanoparticles in a droplet-based microreactor[64]. Importantly, the system affords millisecond time control and also allows the stages of a multistep reaction to be initiated at precise times. In addition, Chan *et al.* have reported the use of microfluidic-droplet reactors for the high-temperature synthesis of CdSe nanoparticles[65], whereas Yen *et al.* have used gas–liquid segmented-flow reactors containing multiple temperature zones for the synthesis of high quality CdSe quantum dots[66] (Fig. 4). In all of these studies, enhanced mixing and reduced residence-time distributions fuelled the improvements in yield and size distribution.

Finally, it should be noted that microfluidic systems can be used to create higher-order nanostructures that are inaccessible via conventional methods. Millman *et al.* recently reported the synthesis of anisotropic particles in static-microdroplet reactors[67]. Droplets with volumes between 500 and 2,000 nl were floated on the surface of a perfluorinated oil. Because droplets can be trapped and manipulated by electrical fields generated by electrode arrays, droplets containing suspensions of

nanoparticles and polymers can be induced to form complex particle structures. For example, 'striped' multilayer particles could be generated from ternary mixtures of gold, fluorescent latex and silica particles, and core–shell particles could be synthesized by encapsulation of dried supraparticles or droplets of aqueous suspension inside polymer shells.

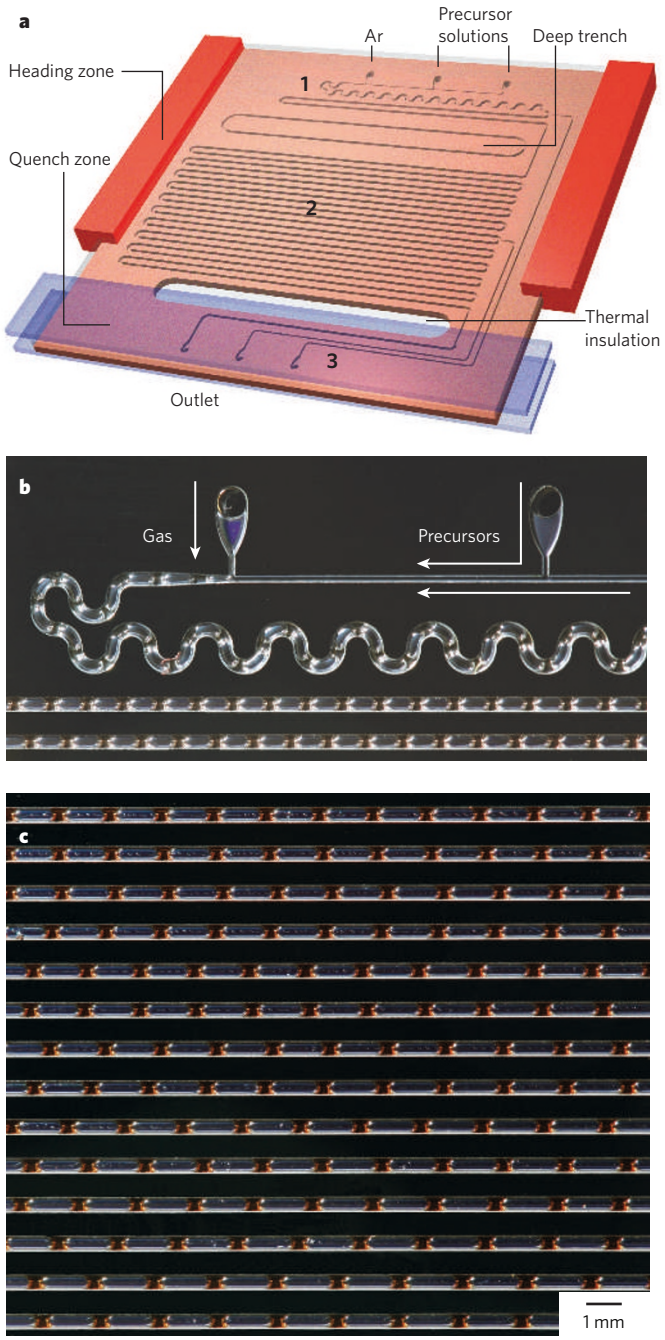

Figure 4 | Microfluidic reactor for nanoparticle production. a, The reactor allows rapid precursor mixing (sector **1**), controlled particle growth (sector **2**) and reaction quenching (sector **3**). The reactor accommodates a ~1-metre-long reaction channel and two shallow side channels for collecting reaction aliquots. A halo etch region allows localization of temperature zones for reaction (>260 °C) and quenching (<70 °C) on the device. Precursor solutions are delivered into the heated section separately, and an argon (Ar) gas stream generates segmented gas–liquid flow. Recirculation within the liquid slugs (droplets) rapidly mixes reagents and initiates the reaction. The reaction is stopped when the fluids enter the cooled outlet region of the device. **b**, **c**, photographs of heated inlets (**b**) and main channel section (**c**). Red segments show the reaction solution; dark segments define Ar gas; T = 260 °C; gas flow rate = 60 μl min^{-1}; liquid flow rate = 30 μl min^{-1}. (Images reproduced, with permission, from ref. 66.)

Microfluidic reactors for DNA amplification

The use of microfluidic systems in synthesis is not confined to small-molecule or nanoparticle synthesis. Indeed, many early applications of microfluidics focused on biological reactions[68–71]. The most investigated biological reaction in microfluidic systems is DNA amplification via the polymerase chain reaction (PCR). This enzyme-catalysed reaction allows any nucleic-acid sequence to be generated in abundance *in vitro*, and has become a fundamental tool in molecular biology[72]. Although simple to implement, PCR in conventional thermal cyclers is slow and inefficient due to large thermal masses associated with instrumentation. To address this issue, many microfabricated devices for PCR have been reported, with performance gains achieved through a reduction in the thermal mass of the system. Effective and rapid PCR in volumes ranging from 1 pl to 50 μl has been performed using various heating mechanisms, including infrared-mediated thermal cycling[73], microwave heating[74], Joule heating[75] and resistive heating[76].

Importantly, the use of micromachining methods has also engendered new approaches to PCR. For example, Krishnan *et al.* have presented an elegant microfluidic system for PCR that relies on the control of thermal convection in a Rayleigh–Bénard cell to provide thermal cycling conditions[77]. Moreover, the widespread adoption of continuous-flow modalities for PCR has been facilitated through the use of microfabricated systems. Continuous-flow PCR (in which a sample is moved continuously through multiple reaction zones held at specific temperatures) has been shown to provide for ultra-fast DNA amplification. Originally described by Kopp *et al.*[78], this approach has yielded the fastest reaction times to date, as the small-volume fluid elements can be heated or cooled to the required temperature within a few milliseconds. More recently, this concept has been extended to create integrated systems for performing reverse transcription and PCR within a single microdevice[79,80].

Finally, it should be noted that, recently, much interest has focused on the creation of highly integrated microfluidic systems for complex biological processing. An elegant example of an integrated monolithic device for DNA amplification was reported by Liu *et al.*[81]. An integrated microfluidic device consisting of mixer elements, fluidic valves and pumps, microchannels, chambers, heaters, and microarray sensors allows for sequential sample preparation (such as cell pre-concentration, purification and cell lysis), PCR, DNA hybridization and electrochemical detection. Moreover, Lagally *et al.* have described the refinement of an integrated genetic-analysis microsystem for PCR and capillary electrophoresis[82]. The microdevice contains microfabricated heaters, temperature sensors and membrane valves to provide controlled sample manipulation and processing of DNA within 200-nl PCR chambers. Using the system, DNA amplification and product sizing could be performed within 10 min, and its utility established through pathogen detection, and genotyping directly from whole *Escherichia coli* and *Staphylococcus aureus* cells.

In general, it is fair to say that microfluidic approaches for PCR have delivered, in terms of the expected advantages with respect to macroscale systems. Indeed, a number of the current generation of commercial thermal cyclers for PCR have embraced the basic tenets of miniaturization. However, the use of microfabricated systems in real-world applications (such as medical diagnostics) is yet to become reality and will ultimately be defined by the ability to create highly integrated microfluidic systems that can handle and process complex biological fluids, and be manufactured at low cost. Fortunately, progress is being made in this crucial area, with contemporary examples of highly-integrated microsystems demonstrating that complex biological processing (such as Sanger DNA sequencing) can be performed at higher speeds and with superior efficiencies than previously achievable[83] (Fig. 5).

Extracting information at the microscale

Compared with the macroscale, microfluidic systems engender significant advantages in terms of speed, throughput, yield, selectivity and control. All are directly facilitated by system downscaling and associated improvements in mass and thermal transfer. Nevertheless, manipulation and processing of samples with instantaneous volumes

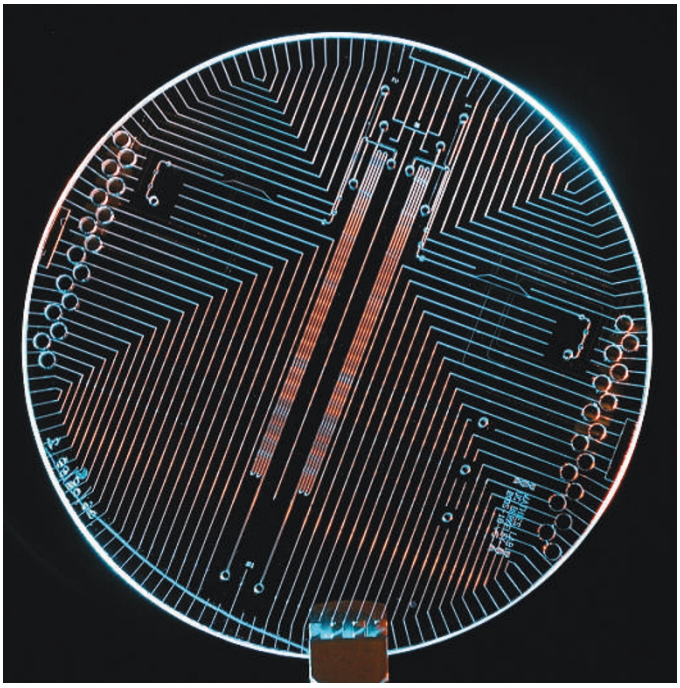

Figure 5 | Integrated microfluidic bioprocessor. A nanolitre-scale microfabricated bioprocessor that integrates thermal cycling, sample purification and capillary electrophoresis for Sanger sequencing. The hybrid glass–poly(dimethylsiloxane) microdevice contains 250-nl reactors, affinity-capture purification chambers, high-performance capillary electrophoresis channels, and pneumatic valves and pumps. Such integration enables complete Sanger sequencing from only 1 fmol of DNA template. (Image reproduced, with permission, from ref. 83.)

ranging from a few picolitres to hundreds of nanolitres provides a significant challenge for analyte detection and identification, and in many ways defines the principal limitations of current microfluidic systems. Detailed evaluation of detection methods for small-volume environments are provided elsewhere[84,85], however, effective detection within microfluidic environments is clearly defined by a close interrelationship of factors such as detector sensitivity, response times, detection limits and information content. Crucially, although microfluidic systems have been shown to be highly effective at generating conditions in which variables such as reagent concentration[86,87], temperature[88] and pH[89] can be controlled with precision, extraction of the available information is normally non-ideal. In other words, although microfluidic reactors generate high-quality chemical information, detection protocols are often inefficient in extracting all available information. For example, we have seen that segmented flow within microfluidic channels allows generation of picolitre-sized droplets (of variable chemical composition) at frequencies in excess of 100 Hz. This means that thousands of individual reactions can be processed in very short times. However, few — if any — studies have successfully exploited this feature, with analytical throughput being defined by the speed at which the detection system can operate.

Despite the challenges of information extraction, some recent reports demonstrate developments in high-throughput chemistry and screening. Ratner *et al.* have reported the use of a microfluidic reaction system to systematically study glycosylation reactions through control of reaction times and temperatures[90]. Using such an approach, optimal temperature/concentration/flow rate protocols were established by in-line high-performance liquid chromatography (HPLC) analysis of the chip effluent. Interestingly, the mannosylation of 2,3,4-tri-O-benzyl-methyl mannoside was achieved in optimal yield at a temperature of –60 °C and a reaction time of 213 s. However, via rapid reaction screening, an almost comparable performance was achieved at a temperature of –35 °C and a reaction time of 26 s. In a similar manner, Leung *et al.* reported ultra-fast screening of reaction conditions within a continuous-flow micromixer using confocal Raman microscopy[91]. The catalytic oxidation of isopropyl alcohol to acetone using tetra-*n*-propylammonium perruthenate and *N*-methylmorpholine-*N*-oxide was assessed as a function of reagent concentrations and residence times. The composition of the reaction effluent was easily determined, and information relating to catalyst/co-oxidant ratios, catalyst turnovers and reaction endpoints extracted. In both studies, the extraction of chemical information in short times, using minimal reagent volumes provides a direct route to rapid synthetic-process optimization, which is not possible in macroscale environments. Very recently, Hatakeyama *et al.* also illustrated rapid optimization of organic reactions within a capillary-based microdroplet reactor[92]. Deacetylation reactions were performed in high-throughput by creating droplet reactors surrounded by a fluorinated carrier fluid, and merging these with a substrate flow. Resulting plugs form, flow into receiving tubing, are stopped, and are isolated by sealing. After incubation, sample is then deposited onto a matrix-assisted laser desorption/ionization (MALDI) plate and analysed by MALDI-mass spectrometry (MALDI-MS) to assess reaction progress. Reaction-condition screening was achieved by screening more than 40 reagents to evaluate reactivity, and then repeating the screen with a more focused reagent set while varying reaction conditions (such as reaction time, solvent and concentration). Although the optimization timescale is limited by the speed of MALDI-MS, the system is simple to implement and able to process reactions on a scale of less than 1 μg per reaction.

Microfluidic factories

Most applications of microfluidic systems in synthesis have focused on the implementation of individual reaction units to demonstrate enhanced performance characteristics compared with macroscale systems. However successful (in terms of yield, purity or speed) these systems are at generating a product, their use in production applications is determined by the ease with which they can be used to generate significant volumes of product in short times. Although characterized by instantaneous volumes in the nanolitre range, microfluidic systems can be configured to achieve such a goal. For example, a system generating product at a concentration of 10% at a flow rate of 200 μl min^{-1} will yield 1.2 ml of product in 1 hour. Therefore, 100 reactors operating in parallel will produce 120 ml h^{-1}, a rate comparable to that of many fine chemical processes. This simple calculation is based on typical examples of reactions with low to moderate yield, and demonstrates that fine-scale processes can be simulated on chip arrays that are within the bounds of current technological development.

A recent example of this concept was provided by Chambers *et al.*, who reported effective scale-out of a steel microfluidic reactor for direct fluorination[93]. Using an array of parallel microchannels coupled by a simple manifold interface to reagent flows, high-purity fluorinated products could be generated at rates of 30 g day^{-1}. Concurrent operation of ten such reactors mimics a small pilot plant operation, running under the same conditions as the laboratory synthesis. Importantly, the system is easy to maintain, operates continuously, requires single-source fluid delivery and operates under safe conditions. In addition, Lee *et al.* recently reported a highly integrated microfluidic system for the direct synthesis of molecular imaging probes used in positron emission tomography (PET)[94]. Although the required volumes are relatively small, the challenge associated with producing radiolabelled chemicals relates to production of highly pure materials within very short timescales (due to the half-life of [^{18}F]fluorine being 110 min). Using a highly integrated microfluidic (Fig. 6), [^{18}F]fluoride concentration, water evaporation, radiofluorination, solvent exchange and hydrolytic deprotection were performed rapidly and with high radio-chemical yield to synthesize 2-deoxy-2-[^{18}F]fluoro-D-glucose. Significantly, product was generated in high enough yield to be used in *in vivo* PET studies.

An elegant approach to 'scale-out' has also been described by Kikutani *et al.*[95]. Simulation of large-scale flows was achieved using a 'pile-up' reactor in which ten glass microchannel circuits were integrated (via thermal bonding) to form a single glass structure. Importantly, although

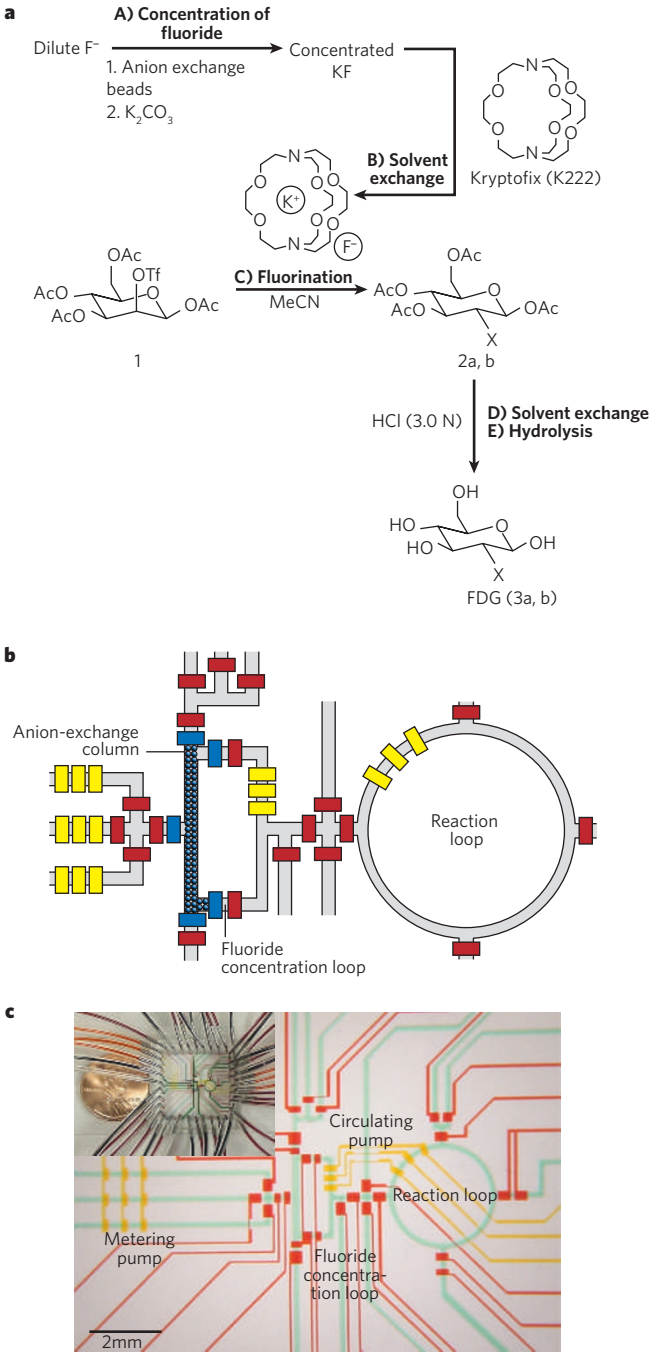

Figure 6 | Schematic representation of a chemical reaction circuit used to synthesize 2-deoxy-2-fluoro-D-glucose. a, Five sequential processes are shown. **A**, Concentration of dilute fluoride ion with an anion-exchange column located in a rectangle-shaped fluoride concentration loop. **B**, Solvent exchange from water to dry MeCN. **C**, Fluorination of the D-mannose triflate precursor 1. X represents ^{18}F (2a and 3a) or ^{19}F (2b and 3b). **D**, Solvent exchange back to water. **E**, Acidic hydrolysis of the fluorinated intermediate 2a (or 2b) in a ring-shaped reaction loop. Nanogram amounts of 2-deoxy-2-fluoro-D-glucose (FDG) (3a, b) define the final product. **b**, The operation of the circuit is controlled by pressure-driven valves, with their delegated responsibilities illustrated by their colours: red, regular valves (for isolation); yellow, pump valves (for fluidic metering circulation); blue, sieve valves (for trapping anion exchange beads in the column module). (Image adapted, with permission, from ref. 94.) **c**, Optical micrograph of the central area of the circuit. The various channels have been loaded with food dyes to help visualize the different components of the microfluidic chip; colours are as in **a**, plus green for fluidic channels. Inset, actual view of the device; a US one-cent coin (diameter 18.9 mm) is shown for comparison. (Image reproduced, with permission, from ref. 94.)

the maximum throughput for the ten-layered pile-up reactor was ten times larger than that of a single microfluidic device, the reaction yield was maintained at 80%. Moreover, a reaction productivity of a few grams per hour scale could be maintained, comparing well with many fine-chemical processes.

After almost a decade of intensive study on the utility of microfluidic systems in chemical production, a few observations can be made. Early research has been successful in demonstrating that many fundamental synthetic transformations can be performed with improved space–time yields, selectivities, reaction residence times and conversions with microfluidics compared with traditional methods. However, application of such systems in industrial environments requires a better understanding of other parameters, such as scalability, facile process control, safety, profitability and operational flexibility[96]. Although there are some examples in commercial production, industry has been slow to embrace microfluidic innovations. This is due in part to limited long-term data on the performance and control of microfluidic reactors in operational environments, and also to cultural factors, such as the widespread investment in and deployment of batch reactors. Indeed, because microfluidic reaction systems can be used to good effect in a number of the stages involved in a chemical process (for example, in compound screening, laboratory-scale process development and production), the extensive implementation of microfluidic factories for chemical production may ultimately be defined by their acceptance as *de facto* tools in these upstream processes, which will, in turn, make them a more natural choice as an industrial tool.

Outlook

The success of microfluidic systems in molecular synthesis is due largely to the exploitation of atypical fluid behaviour in small-volume environments. The fact that fluid properties become increasingly controlled by viscous forces as reaction volumes are reduced dictates that mixing can only be accomplished through diffusion. Nevertheless, at this scale diffusion provides a driver for both rapid and controlled mixing of fluids. Various continuous-flow and batch microfluidic reactors have used these basic ideas to good effect and demonstrated performance characteristics superior to macroscale systems. Although such gains are indisputable, it is less clear how microfluidics will ultimately affect synthesis in both research and industrial environments. Most studies that have used microfluidics in synthesis have either demonstrated the transferral of unit operations from conventional to chip-based formats, or have verified the reality of performance enhancements predicted by theory. This state of affairs defines the initial phase in the lifecycle of microfluidic technology. In many ways, the secondary and critical phase has already begun, whereby microfluidic tools are developed and refined to address and solve fundamental questions. One of the most visible examples of this progression has been the development of segmented-flow droplet reactors. As we have seen, the use of such systems as a basic tool in addressing high-throughput screening and kinetic studies of complex chemical and biological systems defines a totally new approach.

Moreover, as discussed, perhaps the biggest challenge is the efficient extraction and utilization of the vast amounts of information produced. This dictates the development and integration of sensitive detection systems that can process significant amounts of information per unit time. For example, it is expected that the use of high-performance microfluidics will undoubtedly impact the field of catalysis. Droplet systems could be configured to contain a gene and a transcription/translation system that includes all the required ingredients for *in vitro* protein expression. The selection of catalytically-active enzymes can then be performed using molecular biology to generate new catalysts and analytical techniques with which to screen them. The power of such a discovery platform is such that it should be able to screen catalysts at rates up to five orders of magnitude faster than is possible at present.

In conclusion, molecular synthesis in both chemistry and biology has focused, and will continue to focus, on high-throughput experimentation on small samples. Progress in disciplines such as genomics,

proteomics, drug-discovery and high-throughput screening requires new and robust tools that will enable the extraction of enormous amounts of information and, in turn, provide the basis of a better understanding of chemical and biological phenomena. Microfluidics may just provide such tools. ■

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