Microdroplets: A sea of applications?

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The exploitation of microdroplets produced within microfluidic environments has recently emerged as a new and exciting technological platform for applications within the chemical and biological sciences. Interest in microfluidic systems has been stimulated by a range of fundamental features that accompany system miniaturization. Such features include the ability to process and handle small volumes of fluid, improved analytical performance when compared to macroscale analogues, reduced instrumental footprints, low unit cost, facile integration of functional components and the exploitation of atypical fluid dynamics to control molecules in both time and space. Moreover, microfluidic systems that generate and utilize a stream of sub-nanolitre droplets dispersed within an immiscible continuous phase have the added advantage of allowing ultra-high throughput experimentation and being able to mimic conditions similar to that of a single cell (in terms of volume, pH, and salt concentration) thereby compartmentalizing biological and chemical reactions. This review provides an overview of methods for generating, controlling and manipulating droplets. Furthermore, we discuss key fields of use in which such systems may make a significant impact, with particular emphasis on novel applications in the biological and physical sciences.

1. Introduction

During the last two decades the concepts of miniaturization have been applied to the fields of biological and chemical analysis. Of particular note has been the development and application of microfluidic or lab-on-a-chip technology. In simple terms, microfluidics describes the study and development of systems which manipulate, process and control small volumes of fluids (typically on the picolitre to nanolitre scale). Development of microfluidic technology has been stimulated by a range of fundamental features that accompany system miniaturization. These features include the ability to process and handle small volumes of fluid, enhanced analytical performance when compared to macroscale techniques, low unit cost, and perhaps most importantly the ability to access a large number of individual experiments per unit time. In more recent times, the creation of microfluidic systems that are able to efficiently, rapidly and controllably generate sub-nanolitre droplets has defined a new experimental platform for performing a diverse range of chemical and biological processes. Such systems have found application in chemical[1] and biochemical screening,[2] protein crystallisation[3] and enzymatic kinetic assays,[4] and will have a significant impact on other fields such as emulsion based bias-free PCR,[5] DNA sequencing,[6] directed evolution of proteins[7–9] and cell based binding and sorting assays.[10–12]

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At a fundamental level, the use of microdroplets to perform chemistry and biology is appealing since an individual droplet is ideally suited to compartmentalizing and isolating reactants. A significant challenge encountered in single-phase microfluidic systems is being able to suppress dispersion of reacting volumes. Under typical conditions, channel walls impart shear forces on any contained fluid, therefore, under hydrodynamic pressure a parabolic velocity profile is established over the cross-section with fluid velocity zero at the channel walls and maximum at the channel centre (Fig. 1a).[13] The primary implication of such behavior is that a reaction mixture sampled after mixing is formed from an ensemble of volume elements that have spent varying times on-chip. This generates a residence-time distribution that can cause significant variation in the yield, efficiency and product distribution of a given reaction.[14] Accordingly, localization of reagents within discrete droplets is an effective way of eliminating this phenomenon and allowing precise definition of reaction or incubation times in a microbioreactor (Fig. 1b). This precision combined with high droplet throughput and well-defined droplet size renders microdroplet technology an ideal platform for a range of '-omics' approaches where a quantitative readout of a particular process, generated in a rapid and reproducible fashion, is crucial for the ultimate success of an experiment. This is most obvious in the biomedical sciences, where the enormous diversity of genes, proteins and synthetic molecules has impacted new areas such as combinatorial chemistry, proteomics, genomics, and more recently, systems biology, but it is also relevant for more traditional areas such as compound screening in pharmaceutical research. In all these areas, the assay and analysis of large molecular ensembles is key, but the discovery of comprehensive relationships in multidimensional chemical and biological descriptor spaces is limited by the quality and the throughput of the combinatorial

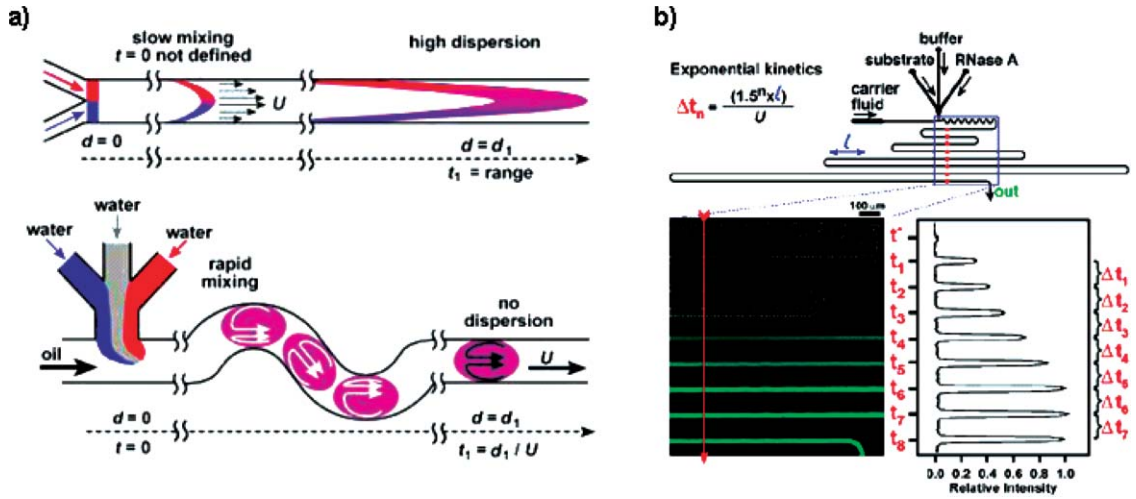

Fig. 1 (a) Comparison of a reaction between two inputs in a pressure driven microfluidic device for a single-phase system (top) and for a droplet-based system (bottom). In a standard microfluidic device the Taylor–Aris dispersion inhibits the distance to time conversion of each reaction, whilst in a microdroplet device the distance (d) a microdroplet traveled is related to the reaction time (t) using the linear flow velocity (U).[2] (b) Measuring millisecond kinetics with microfluidic droplets. By using a T-junction and a three inlet system it is possible to measure the increase of a fluorescent product or enzyme. As the droplets travel along the channel, the reaction residence-time increases. This relation is expressed with Δt_n. With the use of a multiple aqueous inlet system it is possible to vary the concentration of substrate and enzyme leading to the measurement of several concentrations on-chip. The lower image shows a false-colour measurement taken with a CCD camera.[4] Part (a) reproduced with permission from ref. 2. Copyright Wiley-VCH Verlag GmbH and Co. KGaA. Part (b) reproduced with permission from ref. 4. Copyright 2003 American Chemical Society.

experiments. The bottleneck in all high-throughput screening technologies is compartmentalization. Utilization of droplets essentially solves this problem making it conceivable that sample sizes on the order of 10^8 or above can be interrogated in this format. Microdroplets generated in a rapid and reproducible fashion provide a platform for high-throughput screening.[9,15,16]

In the proceeding sections we provide an overview of both bulk and microfluidic approaches for generating droplets. The scope of the review also includes an assessment of droplet control and manipulation mechanisms within microfluidic systems. We also briefly review some of the various applications of microdroplets in the fields of biology, drug discovery and chemistry, whilst providing a critical opinion about how such technologies may (or may not) be implemented in standard laboratory environments. For more extensive details on other facets of microdroplet-based systems the reader is referred to a number of excellent review articles published elsewhere.[8,17–22]

In the early 1980s Batchelder demonstrated that water droplets can be manipulated within a narrow gap filled with insulating fluid and flanked by planar electrodes. This study laid the foundation for the field commonly referred to as digital microfluidics.[23] Although microdroplet flows driven by surface and interfacial tension gradients have been studied over the last century,[24,25] it is only within the last few years that the microfluidics community has renewed its interest in microdroplets and their applications in chemistry and biology. Although of high interest and importance, the field of digital microfluidics is not addressed in detail within the current review, and the reader is directed to an excellent recent review on this subject.[26] Accordingly, herein, microdroplets will refer to droplets with typical length scales in the micron regime and volumes less than one nanolitre.

2. Droplet generation and control

2.1 Generation of droplets

2.1.1 The pipette. If a high-school student was asked to make a microdroplet he or she would probably go into the laboratory, pick up a glass pipette and form many droplets on the table simply by squeezing fluid out of the tip. This method of generating a microdroplet is at the heart of the micropipette concept which has become an invaluable research tool for delivering tiny droplets onto surfaces or cells (Fig. 2).[27–29] A similar approach has recently been used by Jones and co-workers to generate picolitre scale droplets using a piezoelectric ejector. Microlitre volumes of water, deposited from such a device onto a polyimide layer covering coplanar electrodes could

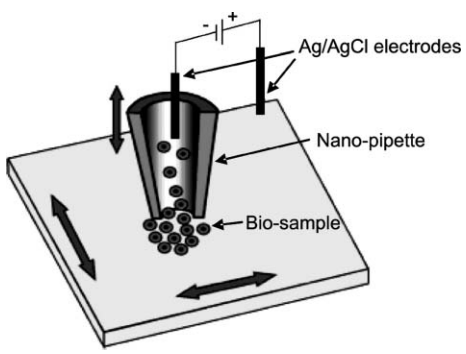

Fig. 2 Schematic of a nanopipette based dispensing technique. A nanopipette (filled with 100 nM solution of DNA or protein) can dispense low volume droplets onto a surface. To do so a voltage between two Ag/AgCl electrodes, one attached to the interior or the pipette, the other to the liquid solution. The droplet size can be fine tuned by varying the ionic current. [146] Reprinted from ref. 29 with permission of Elsevier.

be manipulated, transported and subdivided into droplets as small as ~7 nL by applying a voltage to the tip.[30]

Although such approaches for generating microdroplets are successful in their aims, two key disadvantages exist with reference to their use in chemical and biological analysis. First, droplet transport and manipulation occurs in an open atmosphere leading to rapid evaporation of the liquid sample. Second, the ability to generate and control multiple droplets in an independent fashion is labour intensive and requires complex control architecture.[31] To address both issues, the most commonly used approach for creating droplets involves the use of a secondary oil phase.

2.1.2 Bulk microdroplet generation. An excellent example of bulk microdroplet generation is the entrapment of molecular species during the formation of reverse micelles.[32] In a different fashion Griffiths and Tawfik have developed straightforward protocols to generate femtolitre droplets in bulk oil solutions, negating the use of complicated machinery.[8,9,33,34] The relatively small droplets (with diameters in the μm range) can be likened to bacteria, the basic unit of natural evolution.[7,35] Most of the applications introduced to date using this approach have focused on performing *in vitro* compartmentalization (IVC) as a means for directed evolution of proteins.[36–38] Emulsions on a bulk scale are simple to produce, and can be made highly stable using a variety of oil–surfactant combinations. Indeed, several publications highlight the necessity to control oil–surfactant composition for the efficient emulsification of specific proteins.[7,15,33,34,36,39–43] Such tailoring minimizes unwanted interactions at the droplet interface that might compromise the activity of the biological cargo and prevents loss of substrate or other small molecules due to diffusion into the oil phase. Using this approach, aqueous solutions containing a gene library could be emulsified with a homogenizer in an oil–surfactant mixture within a matter of minutes to produce a water-in-oil (w/o) emulsion containing in excess of 10^{10} droplets per mL. The large excess of droplets over DNA molecules ensures that each microdroplet contains on an average one gene per droplet; providing statistical monoclonality. With a different methodology unilamellar phospholipid vesicles containing a cell-free expression system have been fabricated.[44] Moreover, liposome compartments with femtolitre volumes have been formed and utilized in biological applications.[45,46]

A significant problem encountered when forming droplets *via* bulk methods is the large distribution of microdroplet sizes that typically result. For example, Tawfik and co-workers have reported large size distributions (upwards of 100%) when forming aqueous microdroplets in oil with a mean diameter of 2.6 μm.[7] This high level of size polydispersity can be minimized by using a homogenizer and higher stirring frequencies, but is generally difficult to control.[47,48] Moreover, the high stirring frequencies required result in large shear forces which will often significantly reduce enzymatic activity.[33] To modify the content of droplets after initial emulsification an organic solvent containing the desired compound can be added to the emulsion followed by vortexing. This method has been successfully demonstrated in the delivery of salts and substrates to pre-formed droplets.[34,47,48]

The analysis of the contents of each microdroplet is possible *via* fluorescence activated cell sorting (FACS) if the analyte is fluorescent. As it is impossible to use conventional FACS instruments with a liquid phase of high viscosity (such as oil), re-emulsification of a w/o emulsion is required, generating a water-in-oil-in-water (w/o/w) double emulsion.[15] These double emulsions are complex, soft colloidal systems in which droplets of the dispersed phase themselves contain even smaller droplets. Such systems can be thermodynamically stabilized by a set of lipophilic and hydrophilic surfactants dissolved in the intermediate and external phases, respectively.[33,49] W/o/w compartments have been used as artificial cell-like compartments, and are thus of use in many applications in directed evolution, proteomics, genomics, metabolomics and systems biology.[50–54] However, when using bulk-generated microdroplets in complex biological assays the large degree of polydispersity associated with microdroplet size makes the quantitative analysis of droplet contents (*i.e.* substrate, reagent and product concentration) difficult or impossible.[15,33] Moreover, since droplets formed *via* bulk methods are not ordered in time or space, reactions within individual droplets can only be probed by one endpoint. If large sample sizes ($>10^8$) are to be analyzed, the time required for analysis is on the timescale of hours, so this one endpoint will correspond to a range of time periods passed since the start of the experiment. For a more quantitative assessment of a process in a droplet, the start and end times of an experiment must be rigorously controlled, and for a kinetic analysis more than one time-point is required.[18] Many of these inadequacies could be remedied by the ability to generate, control and handle microdroplets in microfluidic devices. This defines the integration of *in vitro* compartmentalization with microfluidic technology as a major experimental challenge.

2.1.3 Microfluidic droplet generation. In recent years, microfluidic systems have been successfully used to generate multiphase fluids in a variety of formats. Although microdroplets may be formed in microchannels under static conditions,[55] the adoption of continuous flow or pseudo continuous flow microfluidic formats has been shown to provide the most versatile route to high-throughput droplet generation.[56,57]

The generation of microdroplets within microfluidic channels can be initiated through the use of electric fields,[58–60] micro-injectors[61] and needles.[62] However, of particular note are microfluidic systems that exploit flow instabilities between immiscible fluids to generate suspended droplets. Put simply, droplets can spontaneously form when laminar streams of aqueous reagents are injected into an immiscible carrier fluid. The two most common methods for generating droplets in this way are through the use of T-junctions[2,27,63–73] and flow focusing geometries.[51,68,74–77] Within a T-junction device droplets are formed by injecting an aqueous phase perpendicularly into a continuous oil phase.[65,78] Here, microdroplet formation results from induced shear forces within the two phase system.[68,79,80] Flow focusing geometries involve a slightly different configuration, in which a liquid flows in a central channel and a second immiscible liquid flows in two outside channels. The two liquid phases are then impelled to flow through a small orifice that is located downstream of the three channels. The outer fluid applies pressure and viscous stresses that drive the inner fluid into a narrow strand, which then breaks inside or downstream of the orifice (at a position defined by the capillary

number) to form a small droplet.[74,81,82] An example of such a flow focusing configuration is shown in Fig. 3. It should also be noted that microfluidic axisymmetric flow focusing geometries allow generation of droplets with reduced dimensions and size dispersions. Such an approach confines droplets to the central axis of a microchannel and shields them from shear forces and potential wetting upon contact with the channel walls.

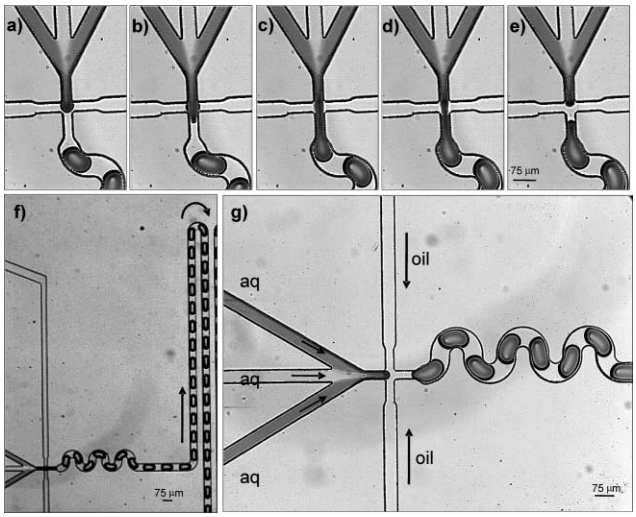

Fig. 3 A pressure driven flow focusing device (FFD) with three aqueous inlets. For better visualization the middle channel delivers colorless phosphate-buffered saline (PBS) only, while other channels contain black and green food dye (10% v/v). As demonstrated here the three aqueous streams do not mix after contact. This phenomenon is known for low Reynolds (*Re*) number flow regimes. Images (a) to (e) shows the procedure for microdroplet formation in more detail. The further the aqueous slug is moved away from the flow focusing point, the more the oil presses against the microdroplet surface until the Rayleigh-Plateau has been overcome and droplets are formed. (f) Shows an overview of a standard FFD device, including a geometric 'wiggle' for fast mixing and loops for increased incubation times. (g) Provides a description of flow lines and liquid input channels. Huebner *et al.* (unpublished results). Images were recorded with a Phantom V7.2 camera.

A key advantage of using microfluidic systems to generate w/o, o/w and w/o/w emulsions is the ability to significantly reduce droplet size distributions when compared to bulk methods.[51,63,65,74,79,83–87] For example, typical microfluidic droplet generators are able to maintain a polydispersity significantly below 3% (measured by diameter), whilst sustaining droplet generation rates in excess of 1 kHz.[79,88] Above this rate droplet production can be parallelized to increase the throughput for industrial purposes. Moreover, cross-contamination between adjacent microdroplets can be essentially eliminated using a separation plug of air or oil between each.[86,87,89]

In broad terms, the properties of droplets produced within microfluidic systems can be controlled by precise variation of volumetric flow rates, fluid viscosities, channel design and the interfacial tension. For example, the interfacial tension can be tuned with the proper choice of oil and surfactant added to the system.[69] The addition of a surfactant is used to control interfacial tension and thus can be used to prevent coalescence of metastable droplets.[69] Furthermore, since microdroplet diameter varies as a function of interfacial tension, droplet size can be decreased by increasing the surfactant concentration within the system.[63,83,90,91] A significant feature of microfluidic droplet generators is the ability to vary the composition of the dispersed phase by utilizing multiple aqueous inlets that combine under laminar flow conditions prior to the droplet formation point. This means that each droplet produced may have a unique composition. This feature provides a direct route towards ultra-fast reaction screening by simply varying the flow rate ratios of all input streams.[3]

2.2 Microfluidic control and manipulation of droplets

Microfluidic systems typically operate within low *Re* number regimes.[92] Although this provides for an environment in which fluidic streams may mix in a controllable fashion, mixing occurs only by diffusive transport rather than through the fast convective processes that dominate in turbulent systems. This can be problematic when processing reactive systems, as the boundary between the two streams or reactants results in a gradient of concentration perpendicular to the flow direction. Moreover, this results in some molecules reacting later (*i.e.* those near the channel walls) than those at the boundary line. This phenomenon is referred to as the Taylor–Aris dispersion. Since fast mixing within microdroplets is achieved for the most part by chaotic advection (Fig. 1a),[4,93–95] or electrowetting,[96] such detrimental properties are overcome, thus allowing precise definition of reaction time within a confined area of the droplet.

The droplets described so far provide a reaction space for an analytical operation, where the reaction is allowed to take place and the outcome is recorded. However, with a variety of post-generation-modifications, such as the fusion and splitting of microdroplets or the isolation of an individual microdroplet, a multifunctional analytical device can be established. This allows for the creation of an ensemble of microdroplet reactors with individuals differing from each other in content (such as pH or reagent concentration). Several groups have investigated and solved many of these engineering tasks and these will be described in the following sections.

The fusion of microdroplets can be achieved using a variety of methods. These include harnessing the difference in the flow velocity of different sized microdroplets,[2] the application of localized electric fields, modification of surface properties[97] and the control of microdroplet velocities by variation of the microchannel geometries.[15,98,99,100] An elegant way to fuse microdroplets is through the implementation of electric forces.[15,67,101,102] For example, microdroplets can be pre-charged on formation, and made to fuse at a desired point of coalescence (Fig. 4).[101] A particularly useful aspect of this approach is the synchronization of the microdroplets, which makes the fusion event highly reproducible. Another feature of microfluidic generation and control of microdroplets is the ability to divide or split them in a facile manner. Splitting of microdroplets can be mediated by a number of mechanisms, including variations in the channel geometry,[2,91,103] variations in both geometry and applied electrical field (Fig. 4),[101,104] pressure,[105] temperature[106] or the localized application of electromagnetic radiation.[68] Finally, it should also be noted that after or between different stages of microdroplet processing it is sometimes necessary to store

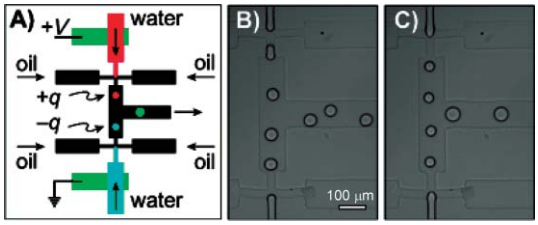

Fig. 4 Coalescence and synchronization of droplets. (A) Describes the overall layout of the microfluidic device. Two flow focusing devices in opposite direction to each other generate and pre-charge droplets. With two inverted electric potentials (red and blue) the droplets are charged negative and positive, respectively. The resulting droplets meet in the middle of the device and fuse. (B) Describes the situation when no electric field is applied. Droplets flow in an asynchronized fashion and no coalescence occurs. (C) At a critical electric field droplets flow in similar manner and fuse.[101] Reproduced with permission from ref. 101. Copyright Wiley-VCH Verlag GmbH and Co. KGaA.

microdroplets, for a period of up to several hours, to allow a particular reaction to take place. To date, this problem has been largely unexplored and needs significant improvement since the on-chip storage capability of slug-like compartments has been shown to only last for several minutes.[2]

Enumerating all the various ways to control and manipulate microdroplets emphasizes the complexity of droplet handling. To realize the concept of a lab-on-a-chip, it is insufficient to demonstrate that droplets can simply be sorted or fused or even produced in parallel. Instead, it must be shown that these events can be integrated to work reliably over extended periods of time and be able to handle a large number of molecular libraries. Moreover, assays may include more complicated control. For example, they may incorporate washing steps, incubation of droplets (allowing cells or *in vitro* expression systems to produce the protein of interest) and presentation to an analytical interface. The construction of a device to meet these requirements will require considerable engineering work. At present, more modest devices represent steps in the right direction. For example, Link and co-workers described a device that included two points of droplet formation, followed by an electrofusion model, and a mixing and delay line. This system was used to discriminate live and dead *Drosophila melanogaster* cells. In many respects this is still a simple device but represents an important stepping stone towards accessing more complex experiments.[107]

3. Application of microfluidic droplet systems in biology

Microdroplets produced in bulk media have been shown to be powerful tools in the evolution of enzymes.[7–9,34,48,53,108] However, as previously discussed droplets generated in this way exhibit a large degree of size (and volume) heterogeneity, and can only be analyzed using endpoint measurement techniques. Tawfik and Griffiths demonstrated the potential of using droplets to improve the reactivity of several enzymes using such an approach.[3,33,32] Furthermore, there is a clear potential to study *in vitro* evolution of proteins and RNAs, cell-free cloning and sequencing, genetics, genomics and proteomics.[8,50] Ismagilov *et al.* have already demonstrated the power of droplet microfluidics in measuring enzyme kinetics. Using such an approach, single-turnover kinetics of the enzyme ribonuclease (RNase) on a millisecond timescale were measured.[4] As the droplets move along the channel an increase in fluorescence was observed. Using several aqueous inlet channels, each supplying a different substrate for the reaction, it was possible to alter the substrate and enzyme concentration "online" through variation in flow rate ratios (Fig. 1b).[4] Similarly, the enzymatic turnover by alkaline phosphatase[109] and the activity of luciferase within droplets have been investigated.[110]

Most methods introduced to date have shown that the general technique can measure the activity of an enzyme on the millisecond scale, without making use of the full potential of the compartmentalization approach, *i.e.* the most published kinetic measurements report on an ensemble average of thousands of droplets. For assays of libraries this approach would not be suitable: no droplet will be identical, so each droplet must be interrogated individually and subsequently sorted to implement selection pressure. An elegant approach for recording data to this end involves the implementation of an epifluorescence laser induced fluorescence detection system, as first reported by Dittrich *et al.*[111] and further extended by Courtois *et al.*[112] In these studies, two aqueous streams (one containing the DNA template and the other containing components for the *in vitro* translation/transcription (IVTT) of green fluorescent protein (GFP)) were merged. Emulsification took place within the PDMS device and the reaction cocktail was then incubated for an extended period of time. Retrieval of thousands of droplets at specific time-points is possible and when combined with sensitive fluorescence detection of the GFP fluorophore protein concentrations can be extracted. These studies demonstrate integration of two modules, droplet formation and incubation. Practically, they provide the instrumental basis for *in vitro* protein evolution experiments with proteins expressed in cell-free systems.[111,112] It should also be noted that careful choice of surfactant formulation was necessary to provide the droplets with enough stability to avoid exchange of their components, fusion, change of volume and aggregation in the reservoir. The selection of an appropriate surfactant is extremely important. Roach *et al.* demonstrated that with proper choice of surfactant it is possible to decrease the likelihood of protein localisation at the interface of the droplet and allow uniform distribution throughout the whole droplet (Fig. 5).[109] For biochemical experiments this becomes crucial as the active enzyme may lose activity due to constraints imposed by the o/w interface. In future, a set-up that allows continuous incubation and retrieval will be highly attractive, but will require a more sophisticated device that can withstand high pressure drops.

Microdroplets are also ideal vessels to encapsulate and contain single or small numbers of biological cells.[113] Systems biologists have already suggested that microfluidic devices can be powerful tools in the understanding of defined networks of cells.[54] Indeed, cells can be co-compartmentalized within microdroplets, and specific dynamics between cells or their metabolites can be probed. Although encapsulation, cultivation and single-cell enzymatic assays have already been successfully conducted in various ways,[105,108,114,115] the control of different cell types and the study of the dynamics of defined populations remain to be demonstrated.

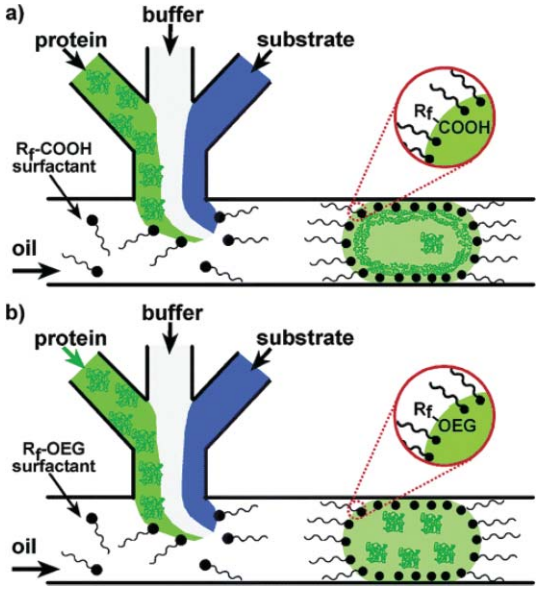

Fig. 5 Demonstration of how surfactants may control and stabilize proteins within aqueous microdroplets. (a) Protein adsorbs to the polar head group droplets of the surfactant based on perfluorotetradecanoic acid (R_f-COOH), whilst in (b) a triethyleneglycol mono[1*H*,1*H*-perfluorooctyl]ether (R_f-OEG) helps to stabilize the protein in its compartment. Importantly, different surfactants must be used for different proteins to ensure a biocompatible interface between the aqueous and oil phases.[109] Reprinted with permission from ref. 109. Copyright 2005 American Chemical Society.

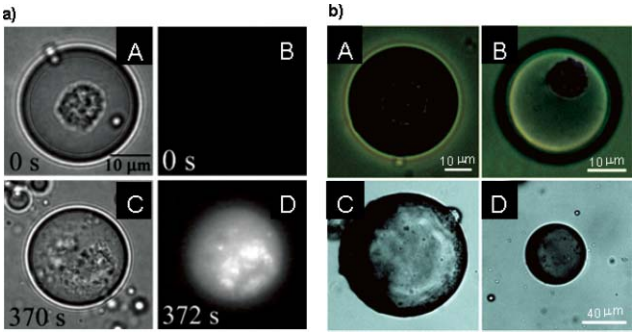

Fig. 6 6a A single droplet-based enzymatic assay. (A) A mast cell was encapsulated in an aqueous microdroplet in soybean oil. The cell contains the fluorogenic substrate fluorescein di-β-D-galactopyranoside (FDG). (B) Because the intracellular enzyme α-galactosidase was physically separated from FDG by the cell membrane minimal fluorescent product within the microdroplet is visible prior to photolysis. (C, D) After laser induced cell lysis (C), the β-galactosidase catalyzes the formation of the product fluorescein, leading to an increased fluorescent signal within the microdroplet (D). The scale bar in (A) applies to all pictures.[12] 6b The behaviour of cells and droplets after freezing. (A and B) Mouse B lymphocytes were encapsulated in droplets containing 50% cell media and 50% phosphate-buffered saline (PBS) and trypan blue to test cell viability. (A) Shows the cell alive and (B) dead. (C) The microdroplet is thawed in silicone oil and (D) in mineral oil.[118] Part (a) reprinted with permission from ref. 12. Copyright 2005 American Chemical Society. Part (b) reprinted with permission from ref. 118. Copyright 2007 American Chemical Society.

For example, the growth of cell colonies has been extended to a single-cell enzymatic assay within a microdroplet. Using optical trapping a single cell was moved to the interface of a T-junction and encapsulated in a droplet.[11] After encapsulation the cell was photolyzed releasing *β*-galactosidase. This enzyme cleaved the quenched fluorogenic substrate within the microdroplet media, leading to an increase of fluorescence over time (Fig. 6). To compartmentalize a cell in a well-defined environment other publications refer to alternative encapsulation methods, such as an extraction method leading to cell viability of several hours[9] or to a photopolymerisation reaction that allowed yeast cells to survive for two days.[116] The beneficial feature of emulsifying cells is that after breaking the cell's structure within the compartmentalized environment the lysate does not diffuse away, thus allowing further investigation of the products. Other methods alter the integrity of the cells in droplets, *i.e.* by lysing, electroporating or freezing.[117] To analyze the influence of thermoelectric manipulation of droplets on-chip Sgro *et al.* froze mouse B lymphocytes in aqueous droplets and performed a viability assay using trypan blue as the indicator (Fig. 6).[118]

The prospect of well-defined droplets as universal reaction vessels is the basis for more rigorous quantification of biological experiments. Accordingly, technical and analytical aspects of system operation will define the ultimate success of high-throughput multiplexing. At the current time it is unclear whether present systems have this potential, since typically the analysis extends only to a series of pictures with droplets containing a cell/protein for demonstration. For example, the rate of droplet formation and the rate of droplet analysis must be synchronized to ensure that neither module becomes the experimental bottleneck in limiting analytical throughput. Recently, the integration of loading and quantitative analysis has been successfully demonstrated in work by Huebner *et al.*[11] In this study, single-cell compartmentalization was coupled to efficient laser induced fluorescence detection to quantify the amount of fluorescent protein production within a cell. Such an approach enabled analysis of in excess of 4×10^6 cells h^{-1} (Fig. 7). Similarly, Srisa-Art *et al.* have recently used confocal spectroscopy and fluorescence resonance energy transfer to probe DNA binding kinetics within individual microdroplets at high speed.[119]

PCR in conventional microfluidic systems[120] takes advantage of performance gains afforded through miniaturization and the operational flexibility inherent in the fluidic design. However, technical challenges remain, for example the reproducibility of amplification and adsorption of polymerase enzyme to channel walls. Extension of PCR to DNA templates confined in droplets would lead to a further reduction in the consumption of reagents, shorter reaction times and more precise amplification due to reduced thermal masses.[5,50] PCR has been conducted in bulk emulsions in several ways. In emulsion PCR (emPCR), DNA or RNA molecules are amplified[18,39] with the amplicon either diffusing freely or bound to beads inside the microdroplet (BEAM-ing).[42] The advantage of emPCR is entirely associated with the concept of compartmentalization. Starting from a single molecule all amplicons in a defined droplet derive from one original template, minimizing bias due to amplification

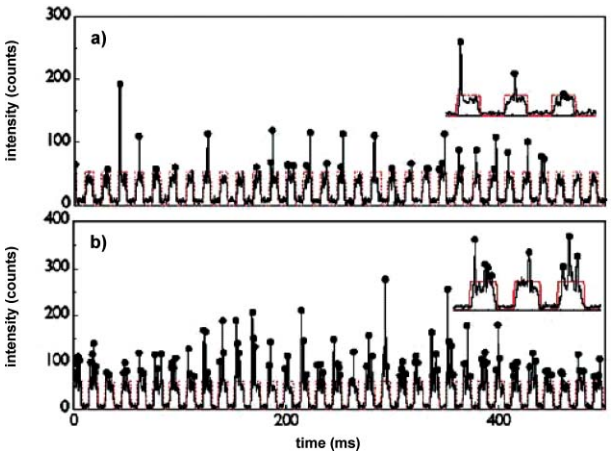

Fig. 7 Analysis of droplets containing a fluorophore expressing *E. coli* cell. With the use of a low fluorescent aqueous medium the microdroplet size and shape can be analyzed. Moreover, peaks with higher intensity are encircled and display the amount of protein. With this method it is possible to measure the cell load of each individual microdroplet. (a) Exhibits the content of droplets using a low number of cells, whilst (b) displays similar data for a high cell load. Control of cell loading is achieved using a T-junction device with two aqueous inlets. One inlet contains the cell suspension, the other only buffer and the fluorescent marker.[11] Reproduced by permission of the Royal Society of Chemistry.

efficiency in bulk (whereas, some templates may overwhelm the final population of DNA).[121,122] In this way, Chabert *et al.*[122] have developed a fully automated PCR thermocycler that incorporates real time detection of the labeled DNA product. However, the droplets (1 μL) are several orders of magnitude larger than those discussed herein (femto- to nanolitres). By contrast Beer *et al.* use much smaller pL droplets that are stopped on-chip by an off-chip valving system for PCR cycling in discontinuous fashion.[123,124] Other applications involving nucleic acids, such as DNA hybridization and PCR in continuous flow slugs[125] or isolated droplets[126] have been published recently.

4. Application of microfluidic droplet systems in drug discovery and chemical synthesis

Screening for products from reactions between probable leads and targets can be facilitated by encapsulating reagents in small containers such as lipsomes.[127] If the composition of each container can be varied and controlled in a systematic and rapid fashion, the use of emulsion droplets as basic tools in small-molecule screening, target identification, small-molecule library generation, lead identification and lead optimization is apparent. As has been discussed, the use of continuous flow microfluidic systems to form droplets is accompanied by the ability to precisely control reagent composition. In other words, droplets of a reproducible size may be generated at high speed and with varying reagent composition. The ability of microfluidic droplets to fine-tune reaction conditions 'on-line' is highly desirable when performing complex processes such as protein crystallisation[128] and molecular evolution.[7]

A number of small-molecule reactions have been performed in liquid plugs within microfluidic systems. These include the nitration of benzene[129] and the halogenation of aromatics[130] and alkenes.[131] Moreover, multi-step reactions such as the synthesis of azo dyes have also been demonstrated.[105]

4.1 Chemical kinetics

Since rapid mixing of the contained fluids is efficient at creating a homogeneous reaction environment, droplet-based microfluidic systems are ideally suited for use in kinetic studies. For example, Ismagilov and co-workers described the use of such a system for measuring the rates of chemical reactions on a millisecond timescale.[132] Specifically, the binding rate of Ca^{2+} to Fluoro-4 (a calcium sensitive dye which becomes strongly fluorescent when combined with Ca^{2+}) was measured. Results showed that the strong fluorescence associated with the mixing of Ca^{2+} and Fluoro-4 inside individual droplets was due to the formation of Fluoro-4 : Ca^{2+}.

4.2 Protein crystallisation

Protein crystallisation is important in enabling structure determination by X-ray diffraction. Proteins may not be available in abundance and therefore a primary goal is to devise a system which miniaturizes the current robotic set-up for crystallisation utilising microtitre plates. Accordingly, microdroplet-based systems provide a versatile platform for use in protein crystallisation, as typical droplets have nanolitre volumes.

Zheng *et al.* demonstrated that by using less than 4 nL of protein solution for each batch, thousands of protein crystallisation conditions could be screened utilising aqueous nanolitre-size droplets.[133] The environment for protein crystallisation could be varied by rapidly and continuously changing the flow rates of the input solutions. Microdroplet pairs created by generating alternating droplets were used to index the composition of the droplets.[3] In other words, an initial microdroplet contains reactants with a second microdroplet holding markers to index the composition of the first microdroplet. For long-term storage, protein crystals inside droplets were transferred from PDMS microchannels to glass capillaries, and X-ray diffraction applied to obtain the diffraction pattern of the protein crystals.[3,134] In addition, the effect of mixing on the nucleation of protein crystals was studied to elucidate the process of protein crystallisation inside droplets.[89,135,136]

4.3 Synthesis of organic molecules

As microreactors, droplets have been utilised for organic synthesis. Deacetylation of ouabain hexaacetate (Ac_6–OUA) was studied by exploiting aqueous droplets moving through a performed cartridge.[137] Easily swollen by many organic solvents, PDMS devices are limited for solvent-based synthesis. Thus, a thiolene-based resin was used to fabricate microfluidic devices to perform organic synthetic reactions within organic-phase droplets.[131] The bromination of alkenes inside benzene droplets was successfully demonstrated using this system. As another example, a synthetic reaction network in the droplets was studied

using the oxidation of Co^{3+} by KH_2PO_4.[94] The reaction was amplified using a two-stage network microfluidic device resulting in a 5000-fold amplification.

4.4 Synthesis of nanoparticles

In recent years, a number of researchers have utilised the unique features of microfluidic reactors to synthesize nanoparticles of defined size and anisotropy. Nanoparticles exhibit optical and electronic properties that are dependent on their size and shape. Such a dependency suggests that 'bottom-up' approaches for nanomaterial synthesis should provide for exceptional control of the physical dimensions of the desired product. Not surprisingly, bottom-up approaches are appealing due to their versatility and ease of use, but for many applications deviations about the mean particle diameter must be <1% to achieve the desired size purity. This is beyond the tolerance of most standard macroscale syntheses, and it is almost always necessary to use some form of post-treatment to extract the desired particle size. Although, continuous flow, single-phase microfluidic reactors have been shown to directly generate high quality nanoparticles of controllable size in short times, more recent studies have tackled the issue of minimizing particle size distributions through the development of segmented-flow or microdroplet reactors. For example, Shestopalov *et al.* demonstrated a two-step chemical synthesis of colloidal CdS and CdS–CdSe core–shell nanoparticles in a droplet-based microreactor.[138] In addition, Chan *et al.* have reported the use of microdroplet reactors for the high-temperature synthesis of CdSe nanoparticles,[136] and Yen and colleagues have used gas–liquid segmented-flow reactors integrating multiple temperature zones for the synthesis of high quality CdSe quantum dots.[139] More recently, the utility of continuous flow droplet reactors has been highlighted by conducting CdS nanoparticle synthesis in a format in which alternating droplets containing Cd^{2+} and S^{2-} ions are fused to form larger droplets containing CdS.[99] In all of these studies, enhanced mixing, minimal residence-time distribution and the ability to control the addition of reagents have directly facilitated enhancements in product yields and size distributions.

4.5 Bead/particle synthesis

The combination of rapid and reproducible microdroplet formation and facile size control microdroplet systems provide a novel route to preparing monodisperse polymer particles. Acrylic monomer droplets were rapidly and reproducibly produced using polyvinyl alcohol (PVA) aqueous solution as a continuous phase.[140] Subsequent polymerisation generated monodisperse particles with a coefficient of variation (CV) below 2%. High-throughput polymerisation of monomeric droplets has also been carried out to obtain polymer particles with various shapes and morphologies.[141] Moreover, microdroplet systems have been applied for molecular imprinting. Uniform molecularly imprinted polymer beads were successfully prepared in a spiral-shaped microchannel using UV polymerisation, which provided a CV below 2% for particle diameters.[142] Parameters affecting the quality of polymer particles such as the polymerisation rate and the geometry of the microfluidic device were studied to attain the desired size and shape of the polymer particles.[83]

4.6 Droplets for microextraction

The extraction of butyl rhodamine B (BRB) by 1-hexanol has been accomplished using a glass device specifically designed to trap organic solvent droplets within microfluidic channels.[143] The BRB solution, which continuously flowed through the channels, was extracted into the organic droplets by phase transfer, after which microdroplet composition was analyzed using LIF. Quantitative extraction using the microdroplet system was also successfully performed to determine the amount of aluminium in water.[144] Aluminium was extracted with 2,2-dihydroxyazobenzene as a metal chelate from a buffer solution into tributyl phosphate droplets. Compared to the conventional extraction method using a separation funnel, the microdroplet extraction was almost two orders of magnitude faster. This technique was applied to environmental analysis, and the results were in agreement with the conventional method. Kralj *et al.* have demonstrated liquid–liquid extraction in a microfluidic device by generating emulsions with large interfacial areas for mass transfer, and subsequently breaking these emulsions using electric fields into easily separated plugs.[145] The result was a transition from disperse to slug flow that can then readily be separated by gravity. Extractions of phenol and *p*-nitrophenol from an aqueous to hexane-surfactant solution served as model systems. Furthermore, it was noted that extraction efficiency is enhanced by reverse micelles resulting from the presence of surfactants.

5. Conclusions and future trends

The studies described in this review clearly indicate that the use of microdroplets as analytical tools in biology and chemistry is an area of huge promise. The ability to reproducibly generate, process and analyze isolated droplets of varying composition and at high frequencies defines a colossal leap in technology for large-scale biological experimentation. Even though such droplet technologies are in their early years, their power is convincing.

The continuing evolution of control architecture to allow more complex intra- and inter-droplet processing, will almost certainly provide the necessary impetus to accelerate microdroplet analysis systems above the mists of engineering to become an indispensable tool in the biological and chemical sciences. The concurrent development of digital microfluidic platforms involving dielectrophoretic control provides a parallel platform towards the understanding of the fundamental mechanisms inside droplets, and it is expected that the marriage of these complementary approaches will enable the creation of novel microfluidic systems for highly sensitive and high-throughput chemical/biological assays. Recently, Huebner *et al.* nicely demonstrated this principle by developing a quantitative cell based enzyme assay within microdroplets.[147]

Considering these facts it is foreseeable that with some engineering the use of microfluidic droplets may lead to standard bench top equipment, thus providing a ***valuable tool for a sea***

of applications ranging from simple biological assays to tissue targeted drug delivery systems.

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References

1 H. Song, M. R. Bringer, J. D. Tice, C. J. Gerdts and R. F. Ismagilov, *Appl. Phys. Lett.*, 2003, **83**, 4664–4666.
2 H. Song, J. D. Tice and R. F. Ismagilov, *Angew Chem., Int. Ed. Engl.*, 2003, **42**, 768–772.
3 B. Zheng, J. D. Tice and R. F. Ismagilov, *Anal. Chem.*, 2004, **76**, 4977–4982.
4 H. Song and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2003, **125**, 14613–14619.
5 R. Williams, S. G. Peisajovich, O. J. Miller, S. Magdassi, D. S. Tawfik and A. D. Griffiths, *Nat. Methods*, 2006, **3**, 545–550.
6 P. Kumeresan, C. J. Yang, S. A. Cronier, R. G. Blazej and R. A. Mathies, *Anal. Chem.*, 2008, **80**(10), 3522–3529.
7 D. S. Tawfik and A. D. Griffiths, *Nat. Biotechnol.*, 1998, **16**, 652–656.
8 A. D. Griffiths and D. S. Tawfik, *Trends Biotechnol.*, 2006, **24**, 395–402.
9 A. Aharoni, A. D. Griffiths and D. S. Tawfik, *Curr. Opin. Chem. Biol.*, 2005, **9**, 210–216.
10 Y. C. Tan, K. Hettiarachchi, M. Siu, Y. R. Pan and A. P. Lee, *J. Am. Chem. Soc.*, 2006, **128**, 5656–5658.
11 A. Huebner, M. Srisa-Art, D. Holt, C. Abell, F. Hollfelder, A. J. deMello and J. B. Edel, *Chem. Commun.*, 2007, 1218–1220.
12 M. He, J. S. Edgar, G. D. Jeffries, R. M. Lorenz, J. P. Shelby and D. T. Chiu, *Anal. Chem.*, 2005, **77**, 1539–1544.
13 S. K. H. A. Stone, *AIChE J.*, 2001, **47**, 1250–1254.
14 S. Krishnadasan, R. J. C. Brown, A. J. deMello and J. C. deMello, *Lab Chip*, 2007, **7**, 1434–1441.
15 K. Bernath, M. Hai, E. Mastrobattista, A. D. Griffiths, S. Magdassi and D. S. Tawfik, *Anal. Biochem.*, 2004, **325**, 151–157.
16 C. M. Dobson, *Nature*, 2004, **432**, 824–828.
17 J. Berthier and P. Silberzan, *Microfluidics for Biotechnology (Microelectromechanical Systems)*, Artech House, Inc., Norwood, MA, USA, 2006.
18 V. Taly, B. T. Kelly and A. D. Griffiths, *ChemBioChem*, 2007, **8**, 263–272.
19 H. Song, D. L. Chen and R. F. Ismagilov, *Angew. Chem., Int. Ed.*, 2006, **45**, 7336–7356.
20 J. Bibette, F. L. Calderon and P. Poulin, *Rep. Prog. Phys.*, 1999, **62**, 969–1033.
21 I. Roland, G. Piel, L. Delattre and B. Evrard, *Int. J. Pharm.*, 2003, **263**, 85–94.
22 K. Welin-Berger and B. Bergenstahl, *Int. J. Pharm.*, 2000, **200**, 249–260.
23 J. S. Batchelder, *Rev. Sci. Instrum.*, 1983, **54**, 300–302.
24 L. E. Scriven and C. V. Sternling, *Nature*, 1960, **187**, 186–188.
25 N. O. Young, J. S. Goldstein and M. J. Block, *J. Fluid Mech.*, 1959, **6**, 350.
26 R. B. Fair, *Microfluid. Nanofluid.*, 2007, **3**, 245–281.
27 L. Ying, A. Bruckbauer, D. Zhou, J. Gorelik, A. Shevchuk, M. Lab, Y. Korchev and D. Klenerman, *Phys. Chem. Chem. Phys.*, 2005, **7**, 2859–2866.
28 A. Meister, M. Liley, J. Brugger, R. Pugin and H. Heinzelmann, *Appl. Phys. Lett.*, 2004, **85**, 6260–6262.
29 H. J. Hwang, K. R. Byun and J. W. Kang, *Physica E (Amsterdam)*, 2004, **23**, 208–216.
30 T. B. Jones, M. Gunji, M. Washizu and M. J. Feldman, *J. Appl. Phys.*, 2001, **89**, 1441–1448.
31 T. Taniguchi, T. Torii and T. Higuchi, *Lab Chip*, 2002, **2**, 19–23.
32 N. L. Klyachko and A. V. Levashov, *Curr. Opin. Colloid Interface Sci.*, 2003, **8**, 179–186.
33 O. J. Miller, K. Bernath, J. J. Agresti, G. Amitai, B. T. Kelly, E. Mastrobattista, V. Taly, S. Magdassi, D. S. Tawfik and A. D. Griffiths, *Nat. Methods*, 2006, **3**, 561–570.
34 A. Aharoni, G. Amitai, K. Bernath, S. Magdassi and D. S. Tawfik, *Chem. Biol.*, 2005, **12**, 1281–1289.
35 V. W. Cornish, *Nature*, 2006, **440**, 156–157.
36 F. J. Ghadessy, J. L. Ong and P. Holliger, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 4552–4557.
37 F. J. Ghadessy and P. Holliger, *Methods Mol. Biol. (Totowa, N. J.)*, 2007, **352**, 237–248.
38 M. d'Abbadie, M. Hofreiter, A. Vaisman, D. Loakes, D. Gasparutto, J. Cadet, R. Woodgate, S. Paabo and P. Holliger, *Nat. Biotechnol.*, 2007, **25**, 939–943.
39 A. Musyanovych, V. Mailander and K. Landfester, *Biomacromolecules*, 2005, **6**, 1824–1828.
40 M. Margulies, M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bemben, J. Berka, M. S. Braverman, Y.-J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. H. Ho, G. P. Irzyk, S. C. Jando, M. L. I. Alenquer, T. P. Jarvie, K. B. Jirage, J.-B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G. T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley and J. M. Rothberg, *Nature*, 2005, **437**, 376–380.
41 T. Kojima, Y. Takei, M. Ohtsuka, Y. Kawarasaki, T. Yamane and H. Nakano, *Nucleic Acids Res.*, 2005, **33**, e150.
42 F. Diehl, M. Li, Y. He, K. W. Kinzler, B. Vogelstein and D. Dressman, *Nat. Methods*, 2006, **3**, 551–559.
43 F. J. Ghadessy and P. Holliger, *Protein Eng., Des. Sel.*, 2004, **17**, 201–204.
44 V. Noireaux and A. Libchaber, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 17669–17674.
45 K. Ishikawa, K. Sato, Y. Shima, I. Urabe and T. Yomo, *FEBS Lett.*, 2004, **576**, 387–390.
46 T. Sunami, K. Sato, T. Matsuura, K. Tsukada, I. Urabe and T. Yomo, *Anal. Biochem.*, 2006, **357**(1), 128–136.
47 J. J. Agresti, B. T. Kelly, A. Jaschke and A. D. Griffiths, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 16170–16175.
48 A. D. Griffiths and D. S. Tawfik, *EMBO J.*, 2003, **22**, 24–35.
49 T. Nisisako, S. Okushima and T. Torii, *Soft Matter*, 2005, **1**, 23–27.
50 J. H. Leamon, D. R. Link, M. Egholm and J. M. Rothberg, *Nat. Methods*, 2006, **3**, 541–543.
51 L. Pier Luigi, F. Francesca and S. Pasquale, *Naturwissenschaften*, 2006, **V93**, 1–13.
52 D. W. D. Pierre-Alain Monnard, *Anat. Rec.*, 2002, **268**, 196–207.
53 H. Leemhuis, V. Stein, A. D. Griffiths and F. Hollfelder, *Curr. Opin. Struct. Biol.*, 2005, **15**, 472–478.
54 D. N. Breslauer, P. J. Lee and L. P. Lee, *Mol. BioSyst.*, 2006, **2**, 97–112.
55 W. Liang, L. Guann-Pyng, X. Wei and B. Mark, *Appl. Phys. Lett.*, 2006, **89**, 144106.
56 S. L. Anna, N. Bontoux and H. A. Stone, *Appl. Phys. Lett.*, 2003, **82**, 364–366.
57 N. Blow, *Nat. Methods*, 2007, **4**, 665–670.
58 H. Mingyan, S. K. Jason and T. C. Daniel, *Appl. Phys. Lett.*, 2005, **87**, 031916.
59 S. J. Kim, Y. A. Song, P. L. Skipper and J. Han, *Anal. Chem.*, 2006, **78**, 8011–8019.
60 O. Ozen, N. Aubry, D. T. Papageorgiou and P. G. Petropoulos, *Phys. Rev. Lett.*, 2006, **96**, 144501–144504.
61 R. M. Lorenz, J. S. Edgar, G. D. M. Jeffries and D. T. Chiu, *Anal. Chem.*, 2006, **78**, 6433–6439.
62 E. Quevedo, J. Steinbacher and D. T. McQuade, *J. Am. Chem. Soc.*, 2005, **127**, 10498–10499.
63 T. Thorsen, R. W. Roberts, F. H. Arnold and S. R. Quake, *Phys. Rev. Lett.*, 2001, **86**, 4163.
64 T. Nisisako, T. Torii and T. Higuchi, *Lab Chip*, 2002, **2**, 24–26.
65 J. D. Tice, H. Song, A. D. Lyon and R. F. Ismagilov, *Langmuir*, 2003, **19**, 9127–9133.

66 J. D. Tice, A. D. Lyon and R. F. Ismagilov, *Anal. Chim. Acta*, 2004, **507**, 73–77.
67 K. Ahn, J. Agresti, H. Chong, M. Marquez and D. A. Weitz, *Appl. Phys. Lett.*, 2006, **88**, 264103–264105.
68 C. N. Baroud, M. R. d. S. Vincent and J.-P. Delville, *Lab Chip*, 2007, **8**, 1029–1033.
69 J. H. Xu, G. S. Luo, S. W. Li and G. G. Chen, *Lab Chip*, 2006, **6**, 131–136.
70 N.-T. Nguyen, S. Lassemono and F. A. Chollet, *Sens. Actuators, B*, 2006, **117**, 431–436.
71 H. Joeska, J. Hengyi, C. H. Erol and C.-W. Justin, *Smart Mater. Struct.*, 2006, **15**, S117.
72 P. Garstecki, M. J. Fuerstman, H. A. Stone and G. M. Whitesides, *Lab Chip*, 2006, **6**, 437–446.
73 J. H. Xu, S. W. Li, J. Tan, W. Y. J. XX and G. S. Luo, *AIChE J.*, 2006, **52**, 3005–3010.
74 L. A. Shelley, B. Nathalie and A. S. Howard, *Appl. Phys. Lett.*, 2003, **82**, 364–366.
75 Z. T. Cygan, J. T. Cabral, K. L. Beers and E. J. Amis, *Langmuir*, 2005, **21**, 3629–3634.
76 P. Garstecki, I. Gitlin, W. DiLuzio, G. M. Whitesides, E. Kumacheva and H. A. Stone, *Appl. Phys. Lett.*, 2004, **85**, 2649–2651.
77 R. Dreyfus, P. Tabeling and H. Willaime, *Phys. Rev. Lett.*, 2003, **90**, 144505.
78 M. Joanicot and A. Ajdari, *Science*, 2005, **309**, 887–888.
79 P. B. Umbanhowar, V. Prasad and D. A. Weitz, *Langmuir*, 2000, **16**, 347–351.
80 J. G. Hagedorn, N. S. Martys and J. F. Douglas, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2004, **69**, 056312.
81 T. Ward, M. Faivre, M. Abkarian and H. A. Stone, *Electrophoresis*, 2005, **26**, 3716–3724.
82 M. M. Dupin, I. Halliday and C. M. Care, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2006, **73**, 055701–055704.
83 S. Xu, Z. Nie, M. Seo, P. Lewis, E. Kumacheva, H. A. Stone, P. Garstecki, D. B. Weibel, I. Gitlin and G. M. Whitesides, *Angew. Chem., Int. Ed. Engl.*, 2005, **44**, 3799.
84 A. S. Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone and D. A. Weitz, *Science*, 2005, **308**, 537–541.
85 E. Lorenceau, A. S. Utada, D. R. Link, G. Cristobal, M. Joanicot and D. A. Weitz, *Langmuir*, 2005, **21**, 9183–9186.
86 T. Nisisako and T. Torii, *Lab Chip*, 2008, **8**, 287–293.
87 W. Li, E. W. K. Young, M. Seo, Z. Nie, P. Garstecki, C. A. Simmons and E. Kumacheva, *Soft Matter*, 2008, **4**, 258–262.
88 M. Joanicot and A. Ajdari, *Science*, 2005, **309**, 887–888.
89 B. Zheng and R. F. Ismagilov, *Angew. Chem., Int. Ed. Engl.*, 2005, **44**, 2520–2523.
90 J. H. Xu, G. S. Luo, G. G. Chen and J. D. Wang, *J. Membr. Sci.*, 2005, **266**, 121–131.
91 D. R. Link, S. L. Anna, D. A. Weitz and H. A. Stone, *Phys. Rev. Lett.*, 2004, **92**, 054503.
92 J. Pihl, M. Karlsson and D. T. Chiu, *Drug Discovery Today*, 2005, **10**, 1377–1383.
93 D. L. Chen, C. J. Gerdts and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2005, **127**, 9672–9673.
94 M. R. Bringer, C. J. Gerdts, H. Song, J. D. Tice and R. F. Ismagilov, *Philos. Trans. R. Soc. London, Ser. A*, 2004, **362**, 1087–1104.
95 R. O. Grigoriev, M. F. Schatz and V. Sharma, *Lab Chip*, 2006, **6**, 1369–1372.
96 M. G. Pollack, A. D. Shenderov and R. B. Fair, *Lab Chip*, 2002, **2**, 96–101.
97 L. M. Fidalgo, C. Abell and W. T. S. Huck, *Lab Chip*, 2007, **7**, 984–986.
98 N. Bremond, A. R. Thiam and J. Bibette, *Phys. Rev. Lett.*, 2008, **100**, 024501–024504.
99 L. H. Hung, K. M. Choi, W. Y. Tseng, Y. C. Tan, K. J. Shea and A. P. Lee, *Lab Chip*, 2006, **6**, 174–178.
100 N. Bremond, A. R. Thiam and J. Bibette, *Phys. Rev. Lett.*, 2008, **100**, 4501–4501.
101 D. R. Link, E. Grasland-Mongrain, A. Duri, F. Sarrazin, Z. Cheng, G. Cristobal, M. Marquez and D. A. Weitz, *Angew. Chem., Int. Ed. Engl.*, 2006, **45**, 2556–2560.
102 M. Chabert, K. D. Dorfman and J. L. Viovy, *Electrophoresis*, 2005, **26**, 3706–3715.
103 D. N. Adamson, D. Mustafi, J. X. Zhang, B. Zheng and R. F. Ismagilov, *Lab Chip*, 2006, **6**, 1178–1186.
104 L. Y. Yeo, R. V. Craster and O. K. Matar, *J. Colloid Interface Sci.*, 2007, **306**, 368–378.
105 G. Axel and K. F. Jenson, *Lab Chip*, 2006, **6**, 1487–1503.
106 T. Ting, Y. Yap, N. Nguyen, T. Wong, J. Chai and L. Yobas, *Appl. Phys. Lett.*, 2006, **89**, Art No. 234101.
107 E. Brouzes, J. Branciforte, M. Twardowski, D. Marran, Y. Suo, Y. Charles, L. Boitard, M. Weiner, J. Rothberg, N. Perrimon and D. Link, *Proceedings of MicroTas 2006 Conference*, 2006, 1043–1045.
108 B. T. Kelly, J.-C. Baret, V. Taly and A. D. Griffiths, *Chem. Commun.*, 2007, 1773–1788.
109 L. S. Roach, H. Song and R. F. Ismagilov, *Anal. Chem.*, 2005, **77**, 785–796.
110 A. Liau, R. Karnik, A. Majumdar and J. H. D. Cate, *Anal. Chem.*, 2005, **77**, 7618–7625.
111 P. S. Dittrich, M. Jahnz and P. Schwille, *ChemBioChem*, 2005, **6**, 811–814.
112 F. Courtois, L. F. Olguin, G. Whyte, D. Bratton, W. T. Huck, C. Abell and F. Hollfelder, *ChemBioChem*, 2008.
113 J. M. Kohler and T. Henkel, *Appl. Microbiol. Biotechnol.*, 2005, **69**, 113–125.
114 K. Martin, T. Henkel, V. Baier, A. Grodrian, T. Schon, M. Roth, J. M. Kohler and J. Metze, *Lab Chip*, 2003, **3**, 202–207.
115 A. Grodrian, J. Metze, T. Henkel, K. Martin, M. Roth and J. M. Kohler, *Biosens. Bioelectron.*, 2004, **19**, 1421–1428.
116 O. Hyun-Jik, K. So-Hyun, B. Ju-Yeoul, S. Gi-Hun and L. Sang-Hoon, *J. Micromech. Microeng.*, 2006, **16**, 285.
117 C. Luo, X. Yang, Q. Fu, M. Sun, Q. Ouyang, Y. Chen and H. Ji, *Electrophoresis*, 2006, **27**, 1977–1983.
118 A. E. Sgro, P. B. Allen and D. T. Chiu, *Anal. Chem.*, 2007, **79**, 4845–4851.
119 M. Srisa-Art, A. J. deMello and J. B. Edel, *Anal. Chem.*, 2007, **79**, 6682–6689.
120 A. J. deMello, *Nature*, 2003, **422**, 28–29.
121 A. T. Woolley, D. Hadley, P. Landre, A. J. deMello, R. A. Mathies and M. A. Northrup, *Anal. Chem.*, 1996, **68**, 4081–4086.
122 M. Chabert, K. D. Dorfman, P. deCremoux, J. Roeraade and J. L. Viovy, *Anal. Chem.*, 2006, **78**, 7722–7728.
123 N. R. Beer, B. J. Hindson, E. K. Wheeler, S. B. Hall, K. A. Rose, I. M. Kennedy and B. W. Colston, *Anal. Chem.*, 2007, **79**, 8471–8475.
124 N. R. Beer, E. K. Wheeler, L. Lee-Houghton, N. Watkins, S. Nasarabadi, N. Hebert, P. Leung, D. W. Arnold, C. G. Bailey and B. W. Colston, *Anal. Chem.*, 2008, **80**(6), 1854–1858.
125 M. Curcio and J. Roeraade, *Anal. Chem.*, 2003, **75**, 1–7.
126 K. D. Dorfman, M. Chabert, J. H. Codarbox, G. Rousseau, P. deCremoux and J. L. Viovy, *Anal. Chem.*, 2005, **77**, 3700–3704.
127 D. T. Chiu, C. F. Wilson, C. Farre, A. Karlsson, S. Nordholm, A. Gaggar, B. P. Modi, A. Moscho, O. Orwar and R. N. Zare, *Science*, 1999, **283**, 1892–1895.
128 P. Kuhn, K. Wilson, M. G. Patch and R. C. Stevens, *Curr. Opin. Chem. Biol.*, 2002, **6**, 704–710.
129 M. A. B. Timothy and S. Sammarco, *AIChE J.*, 1999, **45**, 350–366.
130 N. de Mas, A. Guenther, M. A. Schmidt and K. F. Jensen, *Ind. Eng. Chem. Res.*, 2003, **42**, 698–710.
131 Z. T. Cygan, J. T. Cabral, K. L. Beers and E. J. Amis, *Langmuir*, 2005, **21**, 3629–3634.
132 H. Song, J. D. Tice and R. F. Ismagilov, *Angew. Chem., Int. Ed.*, 2003, **42**, 768–772.
133 B. Zheng, L. S. Roach and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2003, **125**, 11170–11171.
134 M. K. Yadav, C. J. Gerdts, R. Sanishvili, W. W. Smith, L. S. Roach, R. F. Ismagilov, P. Kuhn and R. C. Stevens, *J. Appl. Crystallogr.*, 2005, **38**, 900–905.
135 D. L. Chen, C. J. Gerdts and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2005, **127**, 9672–9673.
136 E. M. Chan, A. P. Alivisatos and R. A. Mathies, *J. Am. Chem. Soc.*, 2005, **127**(40), 13854–13861.
137 T. Hatakeyama, D. L. L. Chen and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2006, **128**, 2518–2519.
138 I. Shestopalov, J. D. Tice and R. F. Ismagilov, *Lab Chip*, 2004, **4**, 316–321.
139 B. K. Yen, A. Gunther, M. A. Schmidt, K. F. Jensen and M. G. Bawendi, *Angew. Chem., Int. Ed. Engl.*, 2005, **44**, 5447–5451.
140 T. Nisisako, T. Torii and T. Higuchi, *Chem. Eng. J. (Amsterdam, Neth.)*, 2004, **101**, 23–29.

141 Z. H. Nie, S. Q. Xu, M. Seo, P. C. Lewis and E. Kumacheva, *J. Am. Chem. Soc.*, 2005, **127**, 8058–8063.
142 M. Zourob, S. Mohr, A. G. Mayes, A. Macaskill, N. Perez-Moral, P. R. Fielden and N. J. Goddard, *Lab Chip*, 2006, **6**, 296–301.
143 H. Chen, Q. Fang, X. F. Yin and Z. L. Fang, *Lab Chip*, 2005, **5**, 719–725.
144 M. Kumemura and T. Korenaga, *Anal. Chim. Acta*, 2006, **558**, 75–79.
145 J. G. Kralj, M. A. Schmidt and K. F. Jensen, *Lab Chip*, 2005, **5**, 531–535.
146 A. Bruckbauer, L. Ying, A. M. Rothery, D. Zhou, A. I. Shevchuk, C. Abell, Y. E. Korchev and D. Klenerman, *J. Am. Chem. Soc.*, 2002, **124**, 8810–8811.
147 A. Huebner, L. F. Olguin, D. Bratton, G. Whyte, W. T. S. Huck, A. J. de Mello, J. B. Edel, C. Abell and F. Hollfelder, *Anal. Chem.*, 2008, **80**(10), 3890–3896.