Chemical and Biological Dynamics Using Droplet-Based Microfluidics

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Abstract

Recent years have witnessed an increased use of droplet-based microfluidic techniques in a wide variety of chemical and biological assays. Nevertheless, obtaining dynamic data from these platforms has remained challenging, as this often requires reading the same droplets (possibly thousands of them) multiple times over a wide range of intervals (from milliseconds to hours). In this review, we introduce the elemental techniques for the formation and manipulation of microfluidic droplets, together with the most recent developments in these areas. We then discuss a wide range of analytical methods that have been successfully adapted for analyte detection in droplets. Finally, we highlight a diversity of studies where droplet-based microfluidic strategies have enabled the characterization of dynamic systems that would otherwise have remained unexplorable.

INTRODUCTION

The concept of miniaturization has proved to be a dominant paradigm in almost all areas of science and technology over the past half century. Most notably, the semiconductor revolution (catalyzed by Jack Kilby's demonstration of the integrated circuit in 1958) has in large part been driven by a continual reduction in both the size and cost of electronic components (1, 2). Such reductions have in turn engendered enormous increases in computing power. Indeed, transistor sizes have decreased by a factor of 10^5 over the past 60 years, with contemporary integrated circuits containing more than 10^9 transistors per cm^2. Significantly, and in addition to the immediate improvements in computational power and cost, such reductions in component size and density also enable entirely new modes of operation and areas of application (1).

In a similar manner, the development of microfluidic technologies over the past 25 years has attempted to transfer related and additional benefits of miniaturization to the fields of chemical and biological experimentation. The rapid acceptance of microfluidic technologies has largely been driven by concomitant advances in the fields of genomics, nanotechnology, proteomics, drug discovery, single-cell analysis, high-throughput screening, and diagnostics, with an identified need to perform rapid measurements on small sample volumes (3). However, at a more fundamental level, microfluidic research has been accelerated by the fact that physical processes can be more easily controlled (in space and time) when instrumental dimensions are reduced to the micron scale. The relevance of such a technology set is significant and characterizes various features that accompany system miniaturization. Such features include the ability to process small volumes of fluid, enhanced analytical performance, reduced instrumental footprints, high analytical throughput, facile integration of functional components within monolithic substrates, and the capacity to exploit atypical fluid behavior to control chemical and biological entities (4, 5).

Unsurprisingly, the efficient manipulation of fluids on femtoliter to nanoliter scales is a nontrivial undertaking. As such, a diversity of dedicated methods has been developed to allow the performance of basic processes, such as fluidic pumping, sample preparation, aliquoting, dilution, concentration, mixing, incubation, and isolation (6). Despite the substantial progress made in this direction, many inherent characteristics of microfluidic systems can be advantageous under certain circumstances but injurious in others. This is the case for continuous flow microfluidic platforms, which leverage the direct scale dependencies of heat and mass transfer while maintaining a high degree of operational and structural simplicity (5). Despite their obvious attractions, continuous-flow formats frequently become less attractive and/or impractical due to issues such as Taylor dispersion, solute surface interactions, cross-contamination, and the need for excessive reagent volumes or relatively long channels (7). Accordingly, alternative methods of fluid manipulation are required if the true potential of system miniaturization is to be fulfilled. To this end, recent years have seen significant interest in the development and application of droplet-based (or segmented-flow) microfluidic systems for chemical and biological experimentation. In basic terms, droplet-based microfluidic systems generate, manipulate, and process discrete droplets contained within an immiscible carrier fluid. They leverage immiscibility to create discrete and isolated volumes that reside and move within a continuous flow. Significantly, these platforms allow for the production of monodisperse droplets at rates in excess of tens of kilohertz and provide for independent control over each droplet in terms of its size, location, and chemical makeup. Critically, the use of droplets in complex chemical and biological processing leverages the ability to perform a range of integrated unit operations in high throughput. Such operations include droplet generation, merging/fusion, sorting, splitting, dilution, storage, and sampling (8, 9). Based on these compelling characteristics, it is unsurprising that droplet-based microfluidic systems are increasingly being used as environments in which to perform a range of biological and chemical assays in an efficient, integrated, and rapid manner.

This review aims to highlight the potentials of droplet-based microfluidic systems in chemical and biological experimentation. Initially, we describe some of the most important and empowering features and unit operations associated with droplet-based systems and then discuss key fields of application in which droplet-based microfluidics have begun to make substantial impact. A particular emphasis is placed on an assessment of where such systems significantly enhance the generation and extraction of dynamical chemical/biological information and where adoption of droplet-based technologies opens up new avenues of research. We direct the interested reader to several other excellent review articles that highlight complementary aspects of droplet-based microfluidics, including physical mechanisms of droplet formation (10), droplet manipulation techniques (11, 12), analytical techniques in droplets (13), single-cell analysis (14), and recent applications of microfluidic droplets (15).

FUNCTIONAL COMPONENTS AND UNIT OPERATIONS

Droplet Generation

Femtoliter- to nanoliter-volume droplets can be generated in a variety of ways within microfluidic systems, but it is important to note that passive strategies for droplet generation have proved to be remarkably straightforward in their implementation and effective in their ability to generate large numbers of user-defined droplets (16). Put simply, passive strategies leverage geometric control of microfluidic intersections to transform arbitrary volumes of fluid into multiple, uniform, and subnanoliter droplets at megahertz rates. The most widely adopted microfluidic constructs for droplet production are the T-junction (or crossflow structure) (17), the flow-focusing geometry (18), and the coflow structure (19) (**Figure 1*a–c***), although refinements of these basic components have also been reported (20, 21). As noted by Collins and coworkers (16), the basic mechanism governing the operation of each of these geometries involves (*a*) the creation of an interface between two coflowing and immiscible fluids and the self-segregation of one of the fluids into (discrete) droplets that are surrounded by the other (continuous) fluid, and (*b*) the identities of the discrete phase (that forms droplets) and the continuous phase (the surrounds droplets) being defined by the surface energies of the two fluids with respect to the channel surface.

The earliest reported use of a microfluidic system for the formation of monodisperse droplet populations was presented by Weitz and coworkers in 2000 (19). Specifically, a coflow of two immiscible phases within a tapered capillary was used to generate droplets (when streamwise forces exceed interfacial tension), whose size is dependent on the capillary tip diameter, continuous phase velocity, extrusion rate, and the viscosities of the two phases. Soon after, Quake and colleagues (17) reported the rapid generation of droplets using crossflow geometry. Here, immiscible fluid streams meet at an angle (normally 90°) to each other, with the fluid of one phase being sheared by a second immiscible phase flowing from the orthogonal channel. Again, droplet size could be controlled by altering the relative flow rates of the two immiscible phases. Finally, the flow-focusing geometry introduced by Anna et al. (18) in 2003 involves the acceleration of concentric but immiscible flows upstream of a small orifice. Such acceleration causes a narrowing (due to pressure and viscous stresses) of the inner fluid thread, which then breaks inside or downstream of the orifice. Significantly, flow-focusing formats provide for control of both droplet size and generation rate over extremely wide ranges.

Despite an overwhelming reliance on droplet generators based around crossflow structures, flow-focusing geometries, or the coflow schemes, other passive methods for droplet generation are highly effective under certain circumstances. These include extrusion over a continuous phase–flooded terrace and step (22) and the use of channel height variations to subject immiscible

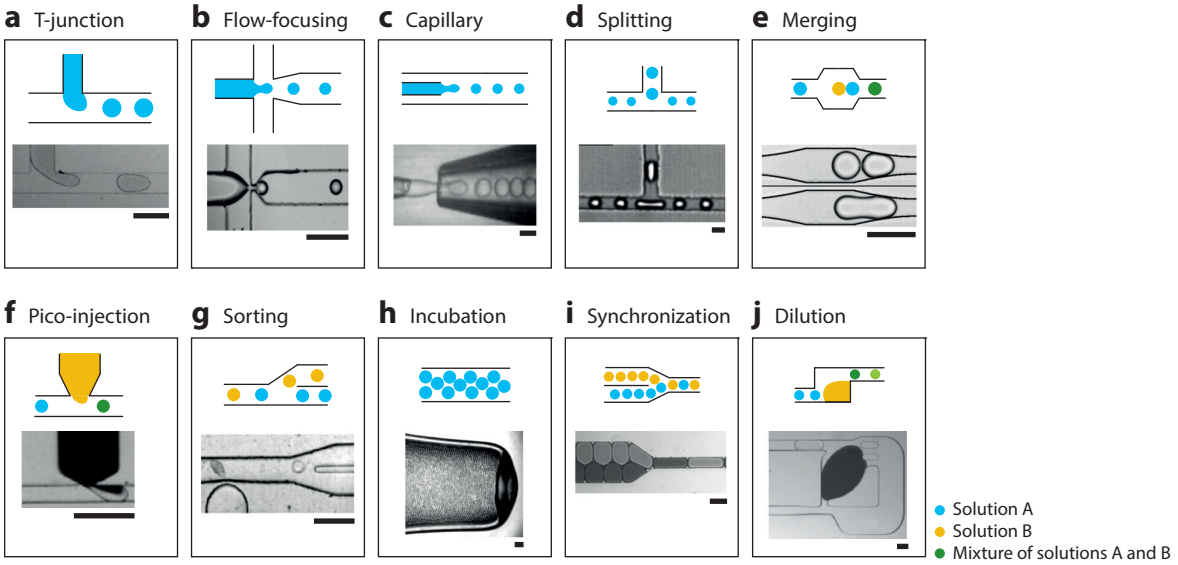

Figure 1

Droplet formation and manipulation (scale bar is 100 μm). The first three panels illustrate common geometries for the production of monodisperse droplets. The (*a*) cross-flow or T-junction geometry relies on the two fluids meeting perpendicularly, whereas (*b*) the flow-focusing geometry involves concentric acceleration of immiscible fluids through a small orifice. Finally, (*c*) a tapered capillary can be inserted into a larger channel, which yields a flow-focusing–like geometry. Droplet manipulation methods are necessary for the translation of complex assays to a microfluidic formats. (*d*,*e*) Splitting and merging droplets can be achieved by controlling channel geometries. Panel *d* adapted with permission from Reference 38. Copyright 2004, American Physical Society. Panel *e* adapted with permission from Reference 40. Copyright 2008, American Physical Society. (*f*) Pico-injection can be used to actively inject a user-defined volume into a passing droplet and is typically triggered using an electrical field. (*g*) Droplets can be sorted by deflection through a variety of forces; here, dielectrophoretic sorting at high frequencies is shown. Panel adapted with permission from Reference 53. Copyright 2015, Royal Society of Chemistry. (*h*) Space-efficient incubation of droplets requires removal of the carrier fluid prior to storage, yielding a tightly packed droplet configuration. (*i*) Low-error passive synchronization of two types of droplets into an alternating configuration can be achieved by self-ordering droplets. (*j*) Serial dilutions of droplets can be achieved by repeatedly merging and resplitting buffer droplets with a stationary mother droplet. Panel adapted with permission from Reference 44. Copyright 2011, Macmillan Publ.: *Nat. Chem.*

interfaces to gradients of confinement (without the need for flow of the external phase) (23). Both approaches are noteworthy, due to their simplicity of operation and ability to robustly produce large populations of monodisperse droplets. Finally, the ability to generate subpicoliter-volume droplets at megahertz frequencies has significant implications for high-throughput single-molecule experimentation. To this end, Shim and coworkers (24) recently presented the use of a constricted flow-focusing geometry to enhance flow velocities during droplet formation (without generating high internal pressures), and via a tip-streaming mechanism in dripping mode, they were able to generate <10 fL-volume droplets at rates well in excess of 10^6 per second. Droplet production rates of several megahertz were also attained by Mittal et al. (25), who used a highly parallelized passive step–emulsification method.

Droplet Storage and Payload Retention

One key feature of droplet-based microfluidic systems is the facility to generate extremely large populations of isolated droplets in very short times. Such parallelization and automation of fluid

handling, combined with the ability to control the chemical/biological payload of individual droplets, make the platform appealing for many biotechnology and large-scale screening applications. As previously noted, droplets are produced in a manner that ensures envelopment by the carrier fluid and separation from both the microchannel surface and adjacent droplets. Accordingly, reactions or assays occurring within droplets can be monitored (through assessment of temporal concentration changes) by following droplets as they move downstream [e.g., using wide-field imaging methods (26)]. Such an approach requires that the reaction kinetics are sufficiently rapid to ensure that any reactions are essentially complete before the droplet under investigation has exited the microfluidic device. In many situations, however, the timescale of the assay or screen is excessively long, and thus droplets need to be stored in a static fashion over periods ranging from hours to weeks (27, 28). Under these conditions, it is critical to ensure that droplet integrity is maintained (i.e., droplets should not merge or split) and that analytes within droplets remain encapsulated and are not transported to the continuous phase or adjacent droplets. Stabilization of droplets is typically achieved using surfactants, which preserve the emulsion and inhibit droplet coalescence by stabilizing the interface between the immiscible phases. Surfactant molecules are normally mixed into the continuous phase, and upon contact, they self-arrange at the interface with the discrete phase. As discussed by Baret (29), surfactant choice is intimately linked to the chemical nature of the phases used and the surface of the containing microchannel.

Due to the need for emulsion stability in a wide range of chemical and biological systems, a variety of surfactants has been developed and used in droplet-based assays. For example, aqueous droplets dispersed in hydrocarbon oils can be efficiently stabilized using commercially available surfactants such as Span80 (17) or Tween20 (30). Nevertheless, due to the widespread adoption of fluorinated oils as carrier fluids (due to their excellent biocompatibility, high gas permeability, and poor solvent capacity for organic compounds) in droplet-based microfluidics (31), fluorosurfactants (perfluoropolyethers containing hydrophilic head groups) offer exceptional long-term stabilization of droplets. In this respect, Weitz and coworkers (28) have reported a novel class of fluorosurfactants synthesized by coupling oligomeric-perfluorinated polyethers with polyethyleneglycol. Such block copolymer surfactants are shown to be highly effective in stabilizing water-in-oil emulsions during droplet formation and incubation and have been used for in vitro translation and single-cell analysis experiments. More recently, the same group (32) reported the synthesis and characterization of polyglycerol-based triblock surfactants (with tailored side chains), which exhibited lower critical micellar concentrations and efficient operation in cell encapsulation and in vitro gene expression studies.

As previously noted, an equally important aspect of droplet stability is the capacity of droplets to effectively retain their contents over long periods of time. Molecular retention is to a lesser or greater extent compromised by mass transport effects through the continuous phase, leading to cross talk between droplets and the equalization of their contents over time. This is especially problematic during storage, when droplets are in close proximity for long periods of time. Although surfactants are extremely effective in stabilizing droplets and indispensable in preventing coalescence, they play a fundamental role in driving cross-talk effects (through micelle formation and molecular interactions between their amphiphilic groups and the molecules contained in the droplets) (33). Crucially, it was recently shown that the surfactant concentration within the continuous phase positively correlates with the permeability of the continuous phase (33). Other parameters that are shown to correlate with droplet permeability include interdroplet distance and flow of the continuous phase around stationary droplets (34). Both studies demonstrate that transport is limited by surfactant-mediated transfer of components through the continuous phase, which occurs more rapidly for closely spaced droplets or under flow conditions (**Figure 2*a***).

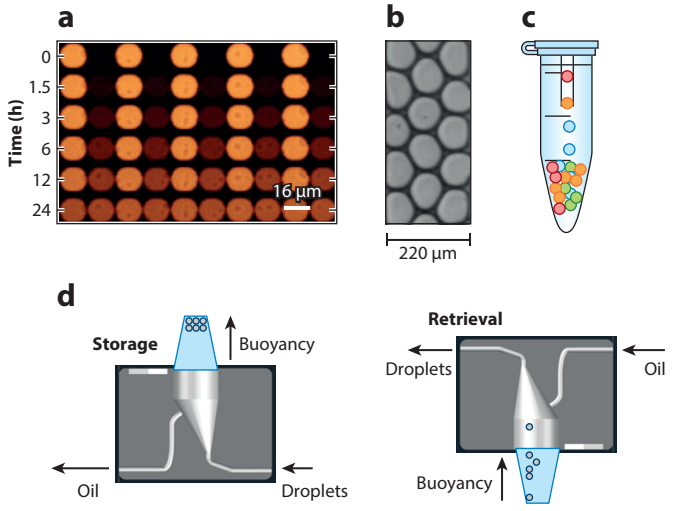

Figure 2

Droplet leakage and storage. (*a*) Gruner et al. (34) reported the transfer of fluorophores between stationary droplets ultimately resulting in homogenous droplet populations. (*b*) Space-efficient storage of droplets requires the removal of the carrier liquid. The resulting tightly packed emulsion exhibits enhanced contact between droplets, which in turn increases the likelihood of interdroplet transfer of analytes. (*c*) Traditionally, droplets are stored in tubes and capped with a third immiscible fluid to prevent evaporation. Nevertheless, droplets adhering to this fluid interface are typically lost during retrieval. Panel adapted with permission from Reference 9. Copyright 2012, Royal Society of Chemistry. (*d*) A simple three-dimensional–printed glass hybrid droplet storage device, which allows for very high droplet retrieval efficiencies. During storage, droplets rise to the top of a silanized glass vial due to buoyancy. Retrieval simply involves turning the device upside-down and applying pressure to the outlet. This simple protocol ensures that droplet losses are <0.5% and can be used for the storage of small and large droplet populations in a reliable manner.

Solid particles can also be used to stabilize emulsions. Pickering emulsions (in which surfactants are replaced by nanoparticles such as colloidal silica) are interesting formulations because they are simple in their construction and yield droplets with a high resistance to coalescence. In recent years, such systems have been used to emulsify microfluidic droplets, minimizing leakage while maintaining droplet stability (35). For example, fluorinated silica nanoparticles are highly efficient at generating Pickering emulsions when using fluorinated oil carrier fluids (35). Nevertheless, it must also be remembered that the inherent stability of Pickering emulsions can also lead to experimental challenges, as nanoparticles are strongly adsorbed at the liquid–liquid interface and thus impede droplet manipulations (such as splitting and fusion).

Finally, as previously noted, many droplet-based experiments require the incubation or storage of droplets over extended time periods. High droplet densities during storage are normally achieved by partial removal of the continuous phase, resulting in tightly packed droplet configurations (**Figure 2*b***). Such an approach ensures that large droplet populations may be assayed but adversely affects both emulsion stability and droplet cross talk (34). Droplets can be incubated in extended channels or tubing, but despite ensuring droplet integrity and minimal cross talk, such techniques do not scale well due to increasing hydrodynamic pressure (36). Alternatively, droplets can be stored at the interface between the continuous phase and a third immiscible phase (**Figure 2*c***). This prevents evaporation of droplets over time by shielding them from ambient air, but it does significantly hinder the retrieval of all droplets due to their strong adherence to this interface. To mitigate such shortcomings, mesofluidic storage chambers can be used for the efficient

storage of large droplet populations. This approach, as exemplified by Dressler and coworkers, allows the facile storage and retrieval of droplets (**Figure 2*d***), with minimal droplet merging or splitting during filling and reinjection. Specifically, a three-dimensional–printed glass hybrid device was used to store the emulsion. During deposition, droplets rose to the top of the silanized glass vial due to buoyancy. Retrieval was enabled by turning the device upside-down and applying pressure to the outlet. The simple protocol combined with the lack of a fluid interface showed the possibility for retrieval of more than 99% of droplets (O. Dressler, M. Lütolf, A. deMello, unpublished data).

Droplet Manipulations

The efficient transferral of chemical and biological workflows to droplet-based microfluidic formats requires the development of a toolbox of unit operations that mirror their macroscale counterparts. Components should perform a given task in a (preferably) passive/automated manner and be readily integrated with other functions that suit the requirements of a specific biological experiment. Such functional components are likely to include modules for droplet generation, fusion, splitting, and sorting; payload mixing, analysis, and dilution; incubation and storage; synchronization (37); and reinjection. Fortunately, over the past decade, numerous microfluidic components have been developed for such tasks and are now available to the experimentalist. A selection of these functional components is now provided (**Figure 1*d–j***).

Aliquoting or droplet sampling is commonly performed by splitting a droplet under study into two or more daughter droplets. Controlling the size of daughter droplets can be achieved directly and passively by forming constricted (nonrelaxed) droplets through variations in channel geometry (38). In this respect, the use of T-junctions or isolated obstacles within the flow path can be used to generate shear forces that overcome surface tension and lead to droplet breakup. Importantly, such geometry-mediated breakup allows the creation of segmented flows with controllable droplet volumes and volume fractions. Conversely, the fusion or merging of two droplets is a fundamental process, affording operations such as reaction initiation/termination, droplet dilution/concentration, and reagent dosing. Numerous techniques for merging droplets within segmented flows have been reported. These are either active [involving the use of electric fields (39)] or passive [leveraging the surface properties or geometry of the flow channel (40)] in nature but most commonly involve bringing droplets into close contact, with subsequent destabilization of the interface between them. Elegant examples of passive droplet merging include the use of channel expansions to destabilize droplet pairs (37) and the exploitation of differences in hydrodynamic resistance of the continuous phase and the surface tension of the discrete phase (through the use of pillar arrays) to controllably merge multiple droplets on millisecond timescales (41). It should also be noted that active approaches for droplet fusion have been realized using a range of external stimuli, including thermocapillary forces (42) and electric fields (43), and provide a degree of dynamic control not open to passive methods.

As noted, the dilution or concentration of droplet contents can also be initiated through controlled droplet merging. Niu et al. (44) reported a simple and elegant method for the creation of droplet concentration gradients by leveraging water–oil hydrodynamic interactions. Specifically, through a series of droplet merging, mixing, and resplitting operations, a nanoliter-volume mother droplet may be combined with a series of smaller buffer droplets to generate a sequence of daughter droplets that define a digital concentration gradient. The addition of reagents to a droplet can be achieved either by droplet coalescence (using the aforementioned methods) or by injecting reagents directly into preformed droplets (a process commonly termed pico-injection) (45). Pico-injection works in a similar fashion to electrically mediated droplet fusion (46), whereby a

pressurized microchannel is used to inject a user-defined volume of reagent into a moving droplet. Put simply, when a droplet passes the pico-injector, electrodes opposite the channel containing the reagent are energized. This destabilizes the water–oil interfaces and allows reagent to enter the droplet (because the pico-injector is maintained at a high pressure). As the droplet moves downstream, it remains connected to the orifice by a narrow bridge of fluid, which eventually breaks and detaches the new droplet. Critically, control of electrode switching allows selective injection of reagent to droplets at kilohertz rates.

Finally, the ability to sort and collect droplets based on their content is a key component of many biochemical assays. Sorting can be achieved passively based on parameters, such as droplet size (47) or deformability (48), but normally requires the rapid assessment of the droplet phenotype followed by the active triggering of the sorting process (49, 50). Sorting strategies typically force passively flowing droplets to switch streamlines using an external perturbation, such as dielectrophoretic force (49), thermocapillarity (42), valves (51), or surface acoustic waves (52). By integrating a gapped channel divider, recent refinements in electrophoretic-based sorting strategies have demonstrated efficient droplet sorting at frequencies up to 30 kHz (53). Such an innovation is significant, because at such high frequencies, sorting is no longer the rate-determining step in the analytical process.

PROBING SMALL VOLUMES: DETECTION IN DROPLETS

The basic requirements of any chemical or biological experiment are the identification and quantitation of relevant species within the assay volume. Detection within the picoliter volumes that characterize microfluidic-based droplets is especially challenging due to the paucity of sample present and the fact that, under normal circumstances, large numbers of droplets are moving at appreciable velocities through the system. Moreover, the study of dynamic processes imposes additional constraints on the detection method, typically requiring multiple measurements of a given droplet over a period of time that may range from a few milliseconds to hundreds of hours. Unsurprisingly, due to their nondestructive nature, high sensitivities, fast response times, and simple integration with chip-based systems, optical methods are by far the most commonly used options for detection in microfluidic formats. The proceeding sections describe some of the most useful optical and nonoptical detection methods for probing segmented flows, with a clear focus on highlighting key advantages and drawbacks associated with each technique.

Fluorescence

A cursory survey of the literature confirms that (laser-induced) fluorescence methods are, by a large margin, the most utilized optical detection techniques in droplet-based microfluidics. The exquisite sensitivity and selectivity of both time-integrated and time-resolved emission spectroscopy are ideally suited to noninvasively probing the picoliter volumes and low-analyte concentrations representative of such droplets. Moreover, the ability to perform fluorescence measurements on submillisecond timescales means that extremely large numbers of droplets (produced at kilohertz frequencies and moving at linear velocities in excess of 1 m/s) can be probed in an efficient and sensitive manner (54). The wide diversity of commercially available fluorescent probes, biological assays based on emissive reporters, and the ability to perform multiplexed (single point or imaged based) detection make fluorescence an ideal detection technique in many applications.

As noted, fluorescence detection is well suited for the extraction of kinetic data. This now well-accepted approach was initially exemplified by Bringer et al. (55), who used time-integrated

fluorescence imaging to quantify mixing dynamics within microfluidic droplets through the emergence of fluorescence due to molecular binding. In addition, Huebner and coworkers (56) elegantly introduced the use of confocal fluorescence spectroscopy to assay protein expression in single *Escherichia coli* cells, which when combined with droplet size measurements, allowed efficient assessment of cell occupancy and growth within picoliter-volume droplets.

The contemporaneous detection of multiple molecular populations can be achieved through a variety of approaches, including the use of multiple point detectors (57) or split-view imaging using charge-coupled device cameras (26). For example, Sjostrom et al. (58) elegantly extracted enzyme kinetics from segmented flows by measuring emission at one wavelength to detect enzyme inhibitor concentrations and emission at a secondary wavelength to quantify enzymatic reaction products (**Figure 3*a***). Moreover, Brouzes et al. (57) screened the cytotoxicity of mitomycin C by simultaneously performing a live-dead assay (using two fluorescence channels) and the concentration of mitomycin C using a third detection channel. A deft development of this basic idea was presented by Zhong and coworkers (59), who used a multiplexed fluorescence readout scheme

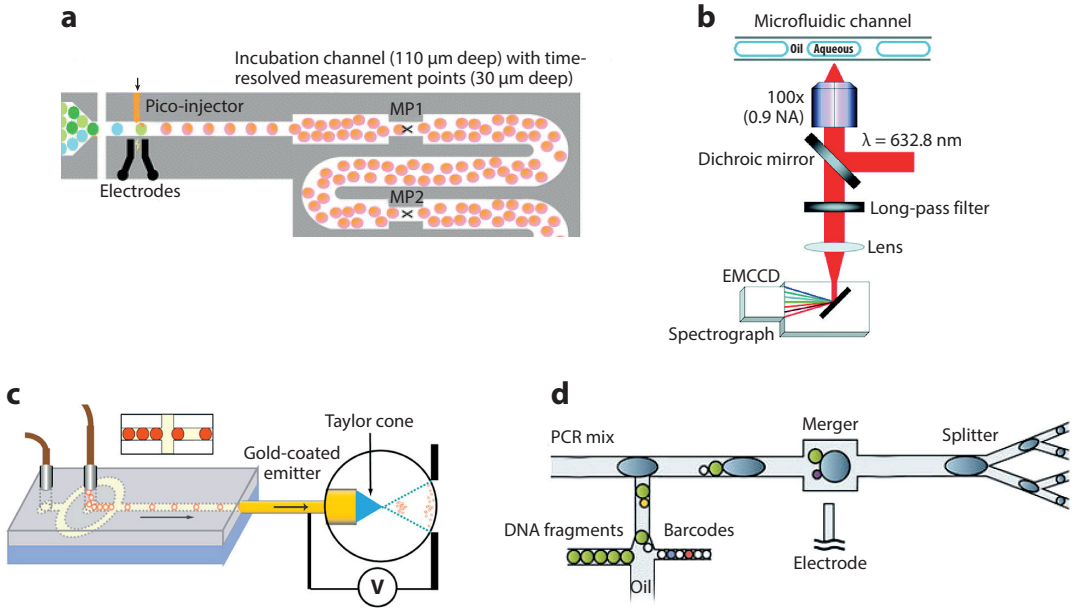

Figure 3

Droplet monitoring. (*a*) Determination of enzyme kinetics using a two-color fluorescence detection scheme. Different concentrations of an enzyme inhibitor are encapsulated in droplets (identified using the first fluorescence channel) and enzyme added via pico-injection. Kinetic constants are determined through quantification of the fluorescent product formed (using the second fluorescence channel). To assay various time points during reaction, the fluorescence intensity is measured at several positions along the channel. Panel adapted with permission from Reference 58. Copyright 2013, Royal Society of Chemistry. (*b*) SERRS in droplets containing silver nanoparticles. Panel adapted with permission from Reference 71. Copyright 2011, American Chemical Society. (*c*) Interfacing droplet microfluidics with an electrospray ionization mass spectrometer. Careful selection of surfactant and carrier oil allows direct injection of microfluidic droplets into the spectrometer without prior phase separation. Panel adapted with permission from Reference 80. Copyright 2013, American Chemical Society. (*d*) Combination of droplets containing random DNA barcodes with droplets containing a cell lysate. During subsequent thermocycling, the barcoded sequences are spliced onto DNA fragments originating from cell lysate. This process allows identification of the cell of origin after sequencing, even if the emulsion is broken and all samples are pooled. Panel adapted with permission from Reference 89 under the terms of the Creative Commons Attribution 4.0 International License, **http://creativecommons.org/licenses/by/4.0**. Abbreviations: EMCCD, electron multiplying charge-coupled device; PCR, polymerase chain reaction; SERRS, surface-enhanced resonance Raman scattering.

in a single digital polymerase chain reaction (PCR) experiment to simultaneously determine 16 separate nucleic acid targets.

The parallel detection of multiple fluorophore populations can also be realized by aligning multiple optical fibers perpendicular to a microfluidic channel (60). Using such a strategy, Cole et al. (60) presented a scheme for multicolor detection of moving droplets by integrating spatially offset optical fibers and a single photodetector. As droplets transit the excitation regions, fluorescent bursts, separated by the time taken to move between excitation regions, are generated and used to perform multicolor detection without the need for spectral filtering of the emission. More complex assays involving fluorescence resonance energy transfer (FRET) (61) or fluorescence lifetime imaging (FLIM) (62) are now routinely used to screen rapid dynamic processes occurring within picoliter-volume droplets. FRET allows distances between sites on macromolecules to be measured by utilizing dipole–dipole interactions between an excited electronic state of a donor fluorophore and the ground state of a proximate acceptor chromophore, with the rate of energy transfer being directly related to the donor–acceptor separation (61). In this respect, Srisa-Art and coworkers (63) successfully employed FRET to study the binding kinetics between streptavidin and biotin within microfluidic droplets on millisecond timescales. FLIM allows the extraction of fluorescence decay times with high spatial resolution and provides an absolute measurement, which when compared to time-integrated signals (intensities), is far less susceptible to artifacts such as nonuniform illumination, scattered light, and excitation intensity variations. Unsurprisingly, FLIM has been used to characterize mixing dynamics in microfluidic droplets on microsecond timescales (64) and is facilitated by the highly controlled and reproducible nature of droplet flows. Interestingly, a combination of FRET and FLIM has also been successfully used to quantify molecular binding in microfluidic droplets, leading to significant reductions in both sample volumes and assay times when compared to bulk measurements (65).

Label-Free Optical Detection

Despite its pervasiveness, fluorescence detection has several drawbacks that limit its usefulness in analytical applications. First, most molecules are nonfluorescent and thus require labeling with fluorescent moieties. Given the complexities of many experimental assays, such preparative steps are undesirable and often interfere with or modify an analyte's normal function. Moreover, multiplexed (multicolor) assays based on time-integrated fluorescence measurements can be challenging due to spectral overlap of fluorophore emission and the requirement for multiple excitation sources and additional optical elements.

To mitigate such drawbacks, a range of label-free optical detection protocols for use in droplet-based microfluidic systems was recently developed. Such techniques typically involve vibrational spectroscopy, a collective term describing both infrared (IR) and Raman spectroscopies. Such techniques are nondestructive and noninvasive and provide information about the molecular composition, structure, and interactions (66).

The inherent chemical specificity and quantitative nature of vibrational spectroscopies make their application in droplet-based systems compelling, but they are somewhat compromised by their reduced sensitivities (a result of their basis on absorption or inelastic scattering processes); this is particularly relevant when applied to dynamic processes occurring within small-volume droplets. That said, recent developments in focal plane array IR detectors (67) and surface-enhanced Raman spectroscopies (68) have opened up new opportunities for the use of vibrational spectroscopies in droplet-based experiments.

Conventional Raman spectroscopic measurements require extended (multiple second) acquisition times when applied to the analysis of biological samples (69). This means that analysis of

nanoliter-volume droplets typically involves averaging the signal over multiple droplets within a segmented flow (69, 70). In contrast, surface-enhanced Raman scattering (SERS) and surface-enhanced resonance Raman scattering (SERRS) can dramatically decrease acquisition times and enhance sensitivity by amplifying Raman signals in excess of eight orders of magnitude (68). The most popular strategy for realizing SERRS in microfluidic droplets involves the incorporation of metal (such as Ag or Au) nanoparticles within droplets (**Figure 3*b***) (71). Due to the large signal enhancements associated with SERRS, sampling frequencies on the submillisecond timescale is possible, which in turn permits single-droplet analysis. Interestingly, the ability to achieve reproducible signal enhancements (a recognized challenge in SERS) is facilitated by the precise control of environmental conditions within droplets. As noted, recent developments in focal plane array detector technology have allowed the efficient integration of IR spectroscopy and IR imaging with droplet-based microfluidics. For example, Chan et al. (72) reported the extraction of Fourier transform (FT)-IR images from picoliter-volume aqueous droplets moving at a speed of 2.5 mm/s and containing millimolar concentrations of lysozyme. Consecutive FT-IR images separated by millisecond time intervals allowed the generation of "chemical movies" at eight frames per second. More recently, Müller and coworkers (73) showed that IR spectra from single droplets could be extracted off-line by directly depositing and drying droplets on a ZnSe prism. This method was successfully used to resolve structural differences between monomeric and aggregated lysozyme protein. Finally, it should also be noted that several studies have reported the successful integration of UV-visible absorption spectroscopy with droplet-based microfluidic systems (74–76).

Mass Spectrometry

Mass spectrometry (MS) is an analytical technique that allows label-free detection of a wide range of analytes. Unsurprisingly, its universality suggests significant application in the analysis of droplet payloads. Unlike optical methods, which are nondestructive in nature, MS analysis requires the transfer of droplets from the microfluidic system to the MS instrument. For example, although simple in principle, interfaces with electrospray ionization MS (ESI-MS) almost always require the integration of a phase separation step, in which the carrier fluid and surfactant [which interfere with electrospray ionization through sequestration of charge carriers and destabilization of the Taylor cone (77)] are removed. That said, a number of reports have shown the successful analysis of droplet payloads by MS. For instance, Fidalgo and coworkers (78) successfully achieved phase separation by coflowing an aqueous stream with a segmented flow and by driving droplets (at a frequency of 0.1 Hz) into the aqueous stream (using an electric field) prior to direct injection into a mass spectrometer. Kelly et al. (79) harnessed differential surface tension properties of channels connected by 3-μm-wide gaps to passively extract droplets from their carrier fluid prior to MS analysis. Using an alternative strategy, Smith and coworkers (80) forewent phase separation by directly injecting a droplet stream into an ESI mass spectrometer, generating spectra for individual droplets at rates exceeding 150 Hz (**Figure 3*c***). However, it should be noted that efficient operation in this study relied on the adoption of specific compositions of carrier fluid and surfactant designed to minimize interference with the injection process.

Finally, droplet flows have also been successfully interfaced with matrix-assisted laser desorption (MALDI)-MS. For example, Pereira and coworkers (81) first reported a liquid chromatography (LC)-MALDI interface based on the formation, processing, and destruction of a segmented flow. The interface consists of a droplet generator to fractionate LC effluent and a deposition probe to transfer sample and matrix onto conventional MALDI-MS targets. Küster et al. (82) also successfully spotted droplets onto a custom microarray plate containing hydrophilic spots. The continuous phase was evaporated, and dried spots were analyzed with MALDI-MS. The approach

allowed for label-free monitoring of an enzymatic reaction, although the deposition procedure required active detection of droplet passage to achieve the correct localization of droplets on the modified plate.

Nuclear Magnetic Resonance and X-Ray Scattering

Despite being the most information-rich analytical technique for molecular structure determination, nuclear magnetic resonance (NMR) spectroscopy is rarely used to analyze mass-limited samples due to its intrinsically low analytical sensitivity. Nonetheless, microcoil NMR probes can probe volumes as small as 200 nL (83), and to this end, Kautz et al. (84) reported the analysis of 2-μL droplet libraries within segmented flows. However, the application of NMR to the analysis of picoliter- to nanoliter-volume droplets is unlikely to find significant impact in next-generation droplet-based systems. Finally, it is worth noting that small-angle X-ray scattering (SAXS) is a formidable technique for the characterization of micro- and nanoscale structures. Despite the need to access suitable beamline sources, SAXS has been used to good effect in the analysis of proteins and nanomaterials in droplets. Zheng et al. (85) first used SAXS in a hybrid chip/capillary system to perform protein crystallization screening and structure analysis in aqueous droplets. More recently, Stehle and coworkers (86) utilized SAXS-based analysis of flowing droplet streams to characterize the size, shape, and concentration of nanoparticles compartmentalized within.

Droplet Barcoding

The ability to identify a single droplet within a large population is central to many of the applications described here. For relatively small populations, this can be achieved by ordering droplets in a linear fashion (87), trapping droplets at specific positions (88), or by labeling droplets with identifiable probes (barcodes) (89). Sequential ordering can be simply achieved by storing droplets inside an extended channel segment or length of tubing. Such an arrangement allows droplets to be reread and identified according to their position relative to the sequence termini (87). Sequential ordering, however, does not scale to large droplet populations, as associated backpressures increase rapidly with the flow path (36). In a similar manner, immobilization of droplets in stationary traps (within larger chambers) does permit the repeated analysis of individual droplets (88, 90, 91), but again, it is limited to a few thousand traps at most. Due to these limitations, several techniques that enable the labeling of individual droplets (barcoding) have been reported in recent years. The most common and straightforward method uses the coencapsulation of multiple fluorophores (leveraging variations in the relative flow rates of the input streams) to spectrally encode droplets and generate uniquely identifiable signatures (92, 93). However, most studies to date have only managed to produce extremely limited numbers (<100) of discrete barcodes (93, 92) and typically generate an unacceptably large number of duplicates [e.g., 80 (92) and 200 (93)]. To address this limitation, we recently developed a programmable system able to generate large populations of droplets, each containing a bespoke barcode. This is achieved by combining the generation of populations of labeled droplets with a barcode reading/sorting technique that captures unique droplets as soon as they are produced. In proof-of-principle experiments, the approach has been successfully used to generate droplet populations containing more than 10^4 droplets with unique fluorescent barcodes using only two fluorophores (O. Dressler, X. Casadevall i Solvas, A. deMello, unpublished data).

Nucleic Acids in Droplets

Although not purely a detection issue, it should be noted that several strategies exist to probe and detect nucleic acid sequences encapsulated within microfluidic droplets. Among the wide range

of implementations of the PCRs in droplets (94–96), techniques for quantitative PCR (qPCR) (97) are especially relevant due to their inherently dynamic nature. Indeed, qPCR can be used to determine the concentration of a target nucleic acid sequence by repeatedly monitoring the emergence of the PCR amplification product. To this end, Beer et al. (98) first showed a variant of qPCR in microfluidic droplets by repeatedly shuttling droplets using off-chip pumps and valves to switch flows. That said, it should be noted that batches of droplets (rather than individual droplets) were assayed, resulting in ensemble-averaged data. Hatch and coworkers (94) have since developed a chip-based digital PCR platform able to monitor in excess of one million droplets in parallel. Due to the ability to monitor individual droplets, the platform could easily be used for qPCR. More recently, Eastburn and coworkers (99) proposed a system that provides for targeted enrichment of nucleic acid sequences using droplet sorting. Droplets containing a target sequence were identified through fluorescence emission from coencapsulated hybridized probes and subsequently sorted.

Several studies have recently reported the integration of next-generation sequencing methods (100) with droplet-based microfluidics. All of these studies have made use of nucleic acid barcodes [introduced directly into the droplet (89), on functionalized beads (101), or encapsulated within hydrogel beads (102)] to label nucleic acids released by a single lysed cell. Droplets are then used to coencapsulate single beads and cells to ensure that a specific barcoded sequence corresponds to a single cell. After reverse transcription, amplification, sequencing, and in silico sequence alignment, the transcriptome of multiple single cells can be determined through the associated barcodes. Despite the low throughput of the sequencing operation, assays can utilize extensive parallelization through concurrent sequencing of all barcoded sequences. This typically requires breaking the emulsion and pooling all samples, thus prohibiting multiple reads of droplets. Originally, Klein et al. (102) identified a population of 10^4 barcodes using RNA-based barcodes, and more recently, Lan and coworkers (89) have barcoded a population of approximately 10^7 droplets using DNA fragments (**Figure 3*d***).

DYNAMIC STUDIES IN MICROFLUIDIC DROPLETS

To elucidate the governing mechanisms of a wide variety of chemical and biological processes, it is often necessary to precisely monitor and characterize their temporal dynamics. Regardless of the associated timescales of such processes (ranging from a few milliseconds to hours or days), droplet-based microfluidic systems can be powerful tools for extracting such information and indeed have already shown utility in extending our understanding of some fundamental problems in chemistry and biology. Some studies in which such benefits have been made manifest are now presented.

Biological Dynamic Processes

Dynamic events affecting microorganisms represent situations where droplet-based screens deliver superior competencies over standard (macroscale) methodologies. For example, the combination of droplet generation followed by droplet trapping allowed Khorshidi and coworkers (103) to automatically track and characterize the behavior and viability of large populations of single cells over extended time periods. Further improvements of traditional cell-based assays can also be effected by leveraging the inherent characteristics of segmented flows. For example, Hofmann et al. (104) cleverly harnessed the physicochemical properties of emulsions (i.e., the osmosis-driven change in droplet size) to quantify chemical reactions and metabolic activity of single cells in a label-free manner. Moreover, Boedicker et al. (105) exploited the confinement properties engendered by microdroplets to study the activation of quorum-sensing pathways in single cells. The authors showed that, simply by virtue of microconfinement, sufficient molecular crowding could

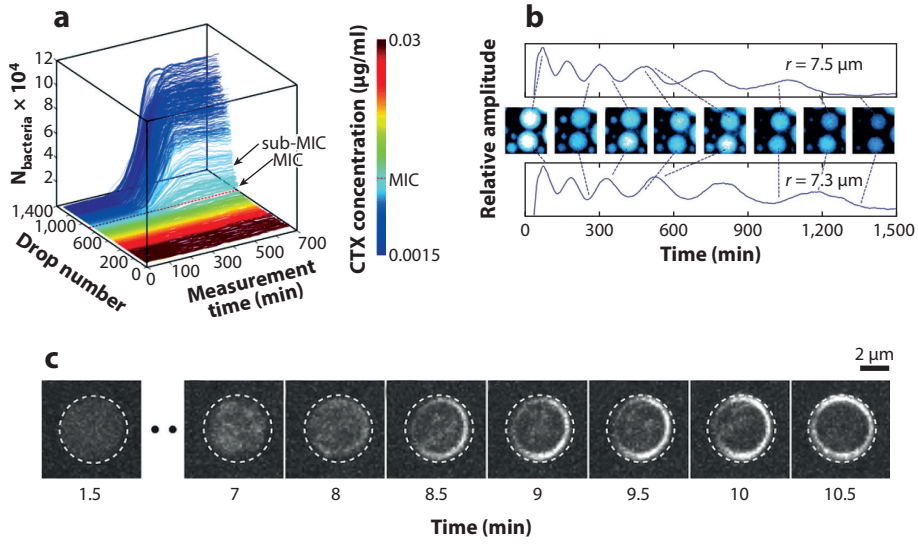

Figure 4

Dynamic biological studies in microfluidic droplets. (*a*) Growth dynamics for bacterial populations encapsulated within millifluidic droplets. Colors indicate the antibiotic (cefotaxime, CTX) concentration in a given droplet. The associated study determines the minimal inhibitory concentration (MIC) of CTX and shows that microfluidic droplets can be a viable tool in the low-cost screening of growth kinetics of microorganisms. Panel adapted with permission from Reference 106. Copyright 2011, Royal Society of Chemistry. (*b*) A transcriptional oscillator encapsulated in droplets, highlighting that encapsulation is critical for the sustained operation of a programmable transcriptional oscillator. Panel adapted with permission from Reference 113. Copyright 2014, Macmillan Publ.: *Nat. Chem*. (*c*) Spontaneous assembly of actomyosin rings within droplets. After initiation of actin polymerization at 0 min, the fibers spontaneously assemble at the equator, despite the analogous process in the cell being guided by spatial regulatory signals. Panel adapted with permission from Reference 119. Copyright 2015, Macmillan Publ.: *Nat. Cell Biol.*

be achieved within droplets to activate a quorum-sensing response in single cells. Significantly, this enabled confirmation that the mechanism responsible for pathway activation is mediated by "diffusion sensing" and "efficiency sensing" rather than by social interactions, as previously believed. In more recent developments, Baraban and coworkers (106) developed a droplet-based technique for the long-term manipulation and screening of more than 1,000 microbial populations (**Figure 4*a***). The method was used to quantify dynamic variables, such as the growth rate of different monoclonal populations of bacteria. Crucially, the authors were able to measure, with unprecedented precision, the minimal inhibitory concentration of an antibiotic by incubating bacteria in droplets across a narrow gradient of antibiotic concentration. Most recently, the same group (107) further applied this methodology to the study of cell-to-cell variability in the growth dynamics of isogenic cell populations and to study the evolution of bacterial populations according to the dynamic emergence of new heritable phenotypes over time (for which the mutations responsible were identified by DNA sequencing). This complex analysis could be achieved by tracking, in parallel, the lineage of descendants of single-cell progenitors. In a similar manner, Jakiela et al. (108) reported the use of a droplet-based system to probe bacterial growth dynamics, which could then be used to study the response, adaptation, and resistance of microorganisms to changing antibiotic environments. Finally, droplet strategies have also been applied to the study of cellular communications across biofilms, a hugely complex environment in which many

cellular signaling mechanisms occur. By encapsulating bacteria into droplets, Weitz and coworkers (109) could dynamically monitor quorum sensing processes between engineered "sender bacteria" encapsulated in one droplet and "receiver bacteria" contained in neighboring compartments.

Synthetic Biology

Synthetic biology aims to recreate biological systems de novo, in what typically follows a bottom-up reconstruction approach. Indeed, by integrating existing biological building blocks, simplified and novel biological circuits can be obtained (110). At a basic level, the implementation of synthetic biology using droplet-based microfluidic platforms makes obvious sense, as one of the fundamental requisites for biological life is compartmentalization (111). Indeed, this has been demonstrated through several studies in which biological processes could only be reproduced by means of droplet compartmentalization. For example, the ability to sustain genetic regulatory oscillations, which are essential mechanisms through which key aspects of cellular function are regulated, is only possible if the synthetic biochemical replicates are adequately encapsulated in droplets (112, 113). For instance, Weitz and coworkers (113) introduced a transcriptional oscillator system into a population of nonuniformly sized droplets to study the stochasticity of the produced oscillation (**Figure 4*b***). Critically, sustained operation of the oscillator was only possible because of compartmentalization, with the bulk oscillatory dynamics quickly converging (dampening) to an average intensity. Furthermore, the authors demonstrated that the degree of compartmentalization (controlled by variations in droplet size) plays a key role in determining the period and dampening of the oscillatory signal. The study and reproduction of biological switches are other important goals of synthetic biology research. To this end, Genot et al. (114) used microfluidic droplets to recreate and study two separate synthetic and nonlinear genetic circuits: a bistable switch and a predator-prey oscillator. By making use of additional droplet-based techniques, such as droplet barcoding, the concentration of input parameters could be extracted in a direct manner, enabling the construction of a detailed map (with over 10^4 data points) of the complete bifurcation diagram.

A pivotal aspect of gene expression, believed to play a key functional role, is its stochastic behavior, which arises from the limited availability of the biomolecular units involved in this process (115). Through encapsulation in droplet-like systems, additional mechanisms affecting gene expression stochasticity have been identified. By using microfluidically produced water-in-oil droplets (116) and coacervates (117), it is possible to synthetically reproduce and study such processes in a crowded environment. Results indicate that additional heterogeneity is obtained when mRNA production rates exceed the diffusion rates of macromolecules.

Synthetic biology techniques have also been used to study and simulate actual cellular processes. In an instructive example of how droplet-based microfluidics have enabled such interfacing, Schwarz-Schilling et al. (118) recently demonstrated the communication between bacteria and a synthetic genetic circuit encapsulated in a droplet via quorum sensing. Alignment of "cell-containing" droplets and "synthetic circuit" droplets in a linear fashion allowed the establishment of a simple model describing spatial differentiation.

Finally, spontaneous biophysical processes that require cell-like compartmentalization to occur are also excellent systems in which the application of droplet techniques can significantly expand current knowledge. Miyazaki and coworkers (119), for example, demonstrated that the assembly of ring-shaped actin bundles (a key step in the cytokinesis cell division program) occurred spontaneously with the support of a droplet-like encapsulation system (**Figure 4*c***). Although the analogous process in the cell is also known to be guided by spatial regulatory signals, equatorial assembly was shown to occur spontaneously within droplets due to the minimization of elastic energy.

Directed Enzyme Evolution

Directed evolution of enzymes aims to mimic the steps of biological evolution and natural selection in the laboratory to evolve species of user-defined function. Typically, a gene library is translated into its associated gene products and screened and/or selected for desired function, while maintaining the link between genotype and phenotype. The most successful phenotypes are then subjected to a round of mutations to generate diversity, with the whole process being repeated multiple times until the desired function is obtained. The high-throughput processing capacity characteristic of droplet-based microfluidic systems combined with the ability to compartmentalize individual units of information (i.e., cells, genes, enzymes) offers significant advantage for the directed evolution of enzymes compared to traditional bulk methodologies (120, 121). Although initial studies exclusively exploited the compartmentalization aspect of bulk emulsions, droplet-based microfluidic methodologies have become more prevalent given their ability to control both the droplet size and payload in a rapid and precise manner. An early example of such superior performance was presented by Baret and coworkers (49), who developed efficient droplet formation and manipulation techniques to encapsulate and sort enzymes based on their dynamic activity. This targeted enrichment approach was then applied to the functional selection of enzymes obtained by several directed evolution techniques, including droplet-based strategies (122) or variants that harness droplet-microfluidic technology to produce hydrogel beads (123) or double emulsions (124). Recently, droplet-based directed evolution was also extended to evolving computationally designed enzyme variants in vitro (125). Another important aspect of droplet-based microfluidics in this respect is that they allow the temporal resolution of dynamic processes through production of droplets in a highly reproducible manner. Such droplets can then be interrogated at different positions during their transit through a microfluidic system, affording the analysis of dynamic catalytic reactions in evolved enzymes (126).

Kinetic Studies

The elucidation of reaction kinetics and mechanisms is another important problem successfully addressed by the application of droplet-based microfluidics. The transferral of kinetic experiments into droplet-based platforms engenders several key advantages with respect to macroscale formats, including the minimization of reactants, significant improvements in the statistical quality of extracted data, and improved control over physicochemical and temporal reaction domains (on millisecond timescales). Moreover, the low reaction volumes typical of microdroplets lead to superior mass and heat transfer, ensuring that reactive processes are kinetically controlled. This, in turn, enables the study of fast (and often hazardous) reactions that are prohibitive in bulk (127).

Extraction of temporal information on reactions occurring within droplets is typically achieved by determining the concentration of reactants and products (within individual droplets) at different positions along the fluidic path (126). For example, early experiments by Song et al. (128) elegantly showed that the kinetics of enzymatic reactions can be extracted from segmented flows, while consuming only minute amounts of reagents (**Figure 5*a***). Despite the fact that the use of segmented flows greatly improves mixing efficiencies, the characteristic mass transfer timescales (of several milliseconds) still required the effects of mixing to be incorporated into the developed kinetic models. Significantly, imaging a large section of the microfluidic system allowed measurement of the average fluorescence intensity produced by multiple droplets passing along the fluidic path, thus simplifying the data acquisition process. Mazutis and coworkers (129) extended previous studies by combining in vitro protein transcription and translation with kinetic analysis (**Figure 5*b***). In this study, a complex multistep assay was performed prior to the extraction of

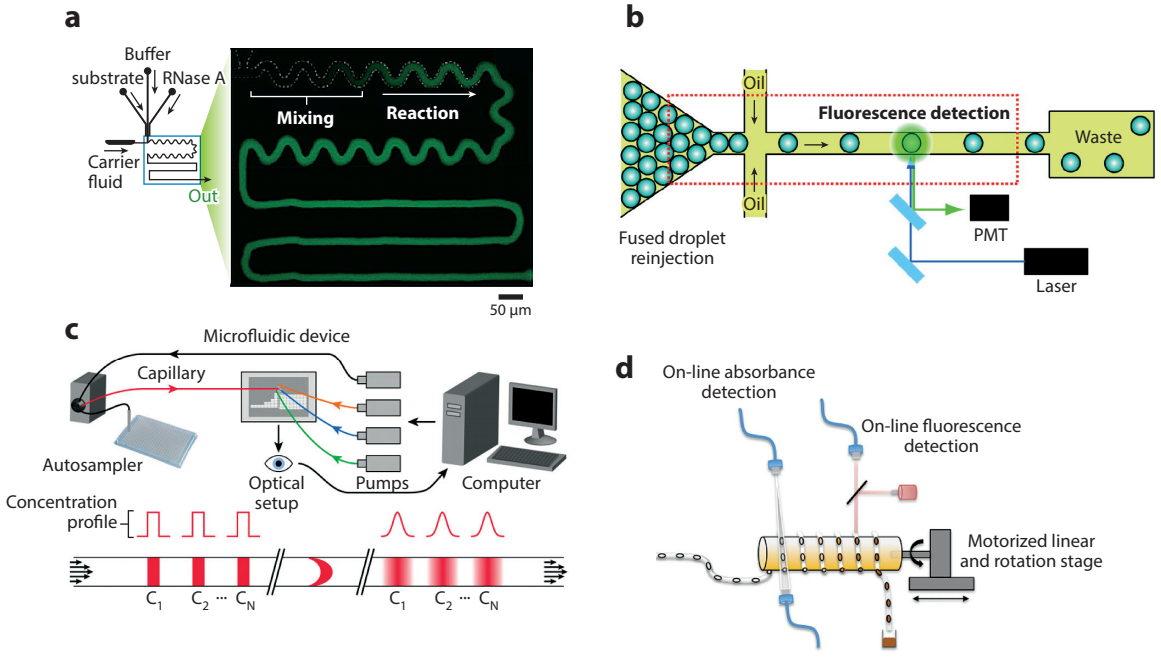

Figure 5

Kinetic measurements in droplets. (*a*) Measurement of millisecond reaction kinetics within microfluidic droplets. Rapid and controllable mixing within droplets enables the study of fast diffusion-limited reactions. Kinetic data are extracted via long-term exposure images (>2 s) and yield signals representative of the average over multiple droplets. Panel adapted with permission from Reference 128. Copyright 2003, American Chemical Society. (*b*) Detection of fluorescence from in vitro translated proteins. DNA is amplified and translated in a droplet-based microfluidic system, allowing the combination of DNA amplification with enzyme activity measurements. Panel adapted with permission from Reference 129. Copyright 2009, American Chemical Society. (*c*) High-resolution dose-response screening in droplets. To increase the number of distinct measurable concentrations, plugs of pure sample are spaced using additional solvent. Plugs are initially dispersed using Taylor-Aris dispersion and subsequently encapsulated into distinct droplets. This allows determination of dose-response curves with unprecedented resolution. (*d*) Integrated platform for the kinetic analysis of PbS quantum dot synthesis. The platform integrates both absorbance and fluorescence detection and allows the user to access different time points of the reaction using a motorized stage. Panel adapted with permission from Reference 135. Copyright 2015, John Wiley & Sons. Abbreviations: PbS, lead sulfide; PMT, photomultiplier tube.

reaction kinetics, highlighting the capacity for integration of functional components and enabling the synthesis and analysis of biological and chemical species within a single platform. Moreover, by combining droplet-based microfluidics with time-resolved fluorescence detection, Maillot et al. (130) temporally resolved the structural relaxation dynamics of bovine serum albumin interacting with Patent blue V. With the addition of droplet-barcoding techniques, multiplexed kinetic measurements have also been demonstrated (93).

To further reduce the reactant volumes needed for large-scale kinetic screens, Fradet and coworkers (131) employed an on-demand droplet formation method to measure reaction dynamics across a wide range of timescales. Although powerful in terms of throughput, disparities with classical measurements were observed due to molecular dynamics at the interface between the two liquid phases. This issue highlights the fact that interfacial phenomena are often central to allowing the efficient application of droplet-based techniques to biological problems. Finally, the ability to resolve dynamics on the single-droplet level is a recognized challenge, as detection in droplet-based experiments has traditionally relied on averaging over large droplet numbers. To

address this limitation, Hess et al. (26) recently developed a stroboscopic imaging technique that enables the efficient probing of individual droplets over extended timescales and the extraction of Michaelis–Menten constants for enzymes incorporated within.

Kinetic analysis of large chemical compound libraries forms an essential part of early-stage drug discovery, and microfluidic methods have been developed to enable such measurements. An example is the generation of dose-response curves, which when using traditional techniques, are built from <10 data points. To this end, Miller and coworkers (132) combined an autosampling technique with a microfluidic device and measured the kinetics of several hundred inhibitors, generating approximately 10,000 data points per compound (**Figure 5*c***). This transformation in experimental precision is achieved by injecting sample pulses into a continuous stream of buffer using a high-performance LC autosampler. Droplets of variable inhibitor concentration could then be generated by harnessing Taylor-Aris dispersion (133) during monophasic transport within a capillary, prior to compartmentalization into picoliter-volume droplets using an oil-based carrier fluid.

Despite the prevalence of fluorescence methods for kinetic exploration, it should be noted that other measurement techniques have also been developed. For example, potentiometric measurement of free Mg^{2+} ions was applied to determine binding kinetics between Mg^{2+} and RNA molecules (134). Hassan and coworkers (74) have also reported the development of a custom flow cell to perform multipoint absorbance measurements of droplets in flow, which in turn, could be used to quantify reaction rates of the enzymatic degradation of glucose.

Finally, the application of droplet-based microfluidics has proven crucial in the analysis of the reaction dynamics and mechanisms associated with nanomaterial syntheses. Despite the abundance of synthetic routes for their production, experimental validation of theoretical models for nanoparticle nucleation and growth has rarely been addressed due to the difficulty in precisely extracting kinetic data on short timescales using conventional methods. To address such oversight, Lignos et al. (135, 136) harnessed the exquisite control over reaction times and mixing rates inherent in droplet-based systems to evaluate the early-time kinetics (<1 s) of lead sulfide quantum dot formation, obtaining new insights on the early-stage mechanism of nucleation of metal halide nanocrystals (**Figure 5*d***). Subsequently, Maceiczyk and colleagues (137) used droplet microfluidics to expand the current understanding of nanoparticle nucleation and growth kinetics by including a diffusion term that describes the influence of surfactant concentration on the synthesis of CdSe quantum dots. Finally, Abolhasani et al. (138) recently reported the use of a droplet-based oscillatory platform that removed the residence time limitations associated with continuous multiphase strategies to spectrally characterize II–VI and III–V quantum dots, providing further insights into the kinetics and mechanisms of their nucleation and growth.

CONCLUSIONS

Since the first proof-of-concept experiments (17–19), droplet-based microfluidic technologies have become an increasingly important and powerful tool in biological experimentation. Significantly, a large variety of droplet-based platforms and tools is now being used in a routine manner by researchers from a wide range of scientific disciplines. Despite this growing popularity, however, droplet-based techniques have found a more limited application in the study of dynamic systems, primarily due to the lack of appropriate methodologies to label and identify large numbers of individual droplets and because of intrinsic issues related to phase transfer. That said, the studies and innovations described herein clearly suggest that a diversity of methods is now available for the generation, manipulation, and detection of droplets. Moreover, these core technologies are being increasingly applied to the study of dynamic (chemical and biological) processes

that were hitherto inaccessible. Such advancements have been engendered through the essential properties of microemulsions, most notably molecular confinement and interfacial effects. These techniques have realized some remarkable successes in single-cell sequencing (89, 101, 102) and synthetic biology, for example. Nonetheless, many challenges (and indeed opportunities) await as droplet-based microfluidic systems transition from proof-of-concept platforms to integrated and commercialized instruments.

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