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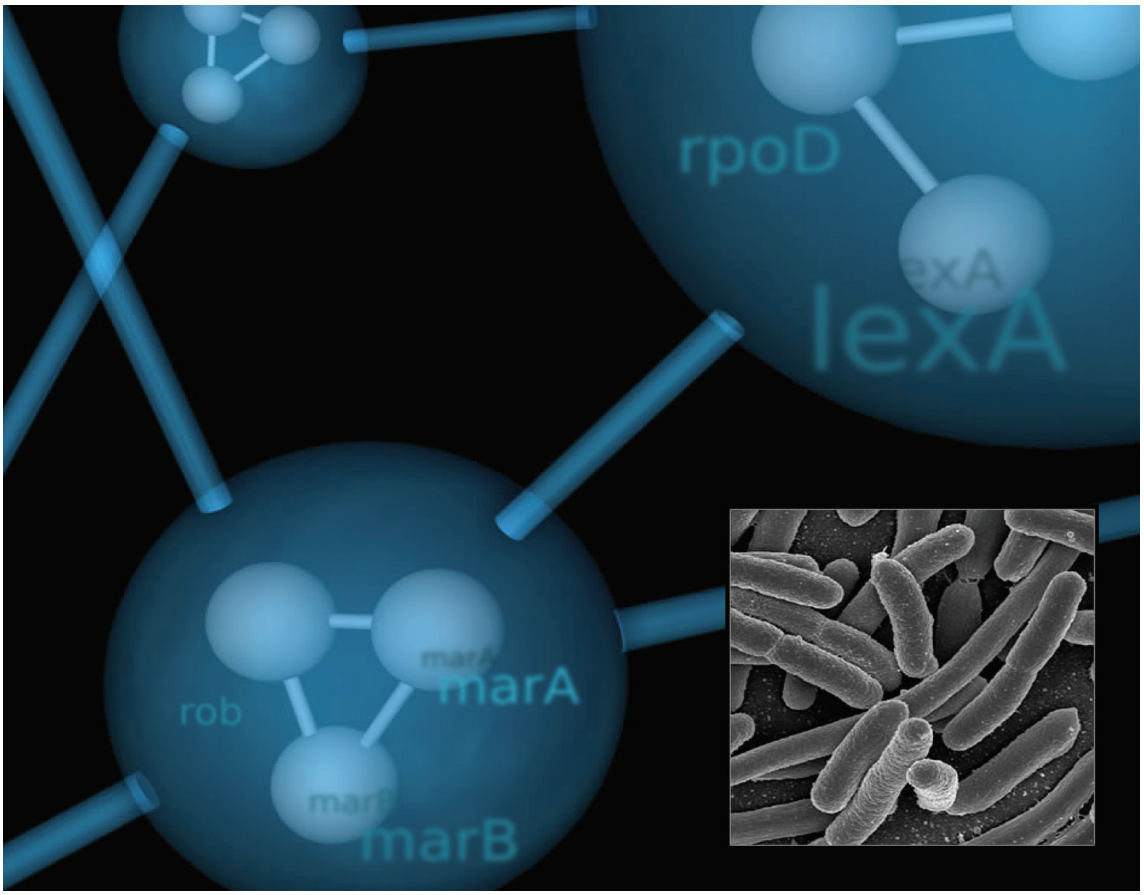

The potential of microfluidic water-in-oil droplets in experimental biology†

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The comprehensive characterisation of complex parameter space in '-omics' technologies requires high-throughput systems. *In vitro* compartmentalisation of reactions in water-in-oil droplets combines the necessary ability to carry out large numbers of experiments under controlled conditions with quantitative readout, and has recently advanced towards automation by generating droplets in microfluidic devices. Some approaches based on these principles are already familiar (*e.g.* emulsion PCR for sequencing), others, including directed evolution or cell-based assays, are in advanced stages of development—and proof-of-principle experiments are appearing for a whole range of applications in diagnostics, cellomics, proteomics, drug discovery and systems and synthetic biology. This review describes the current state-of-the-art, notes salient features of successful experiments and extrapolates in the direction of more highly integrated systems.

Introduction

Challenges in experimental biology

Experimental challenges in contemporary biology increasingly demand high-throughput experiments, to provide information on large parameter spaces from cell populations to DNA, protein or small molecule libraries. Practically, such a format should be highly economical, involving minimal sample consumption of potentially precious biological reagents. Analytically the study of single genes, cells or even proteins is desired to resolve experiments at this level in contrast to conventional bulk experiments. Furthermore these large-scale experiments are to be conducted under tightly controlled conditions, with a reliable, quantitative readout.

The potential of microfluidic droplets

In vitro compartmentalisation—a technology originally developed by Griffiths and Tawfik for directed *in vitro* evolution[1]—provides a system to address these requirements. Compartmentalisation of individual samples in aqueous droplets dispersed in an oil phase is becoming a powerful method for high-throughput assays in chemistry and biology.[2–5] Here the droplet is the equivalent of the test tube,[6] with droplet volumes in the femto- to nanolitre range, up to 10^{10} droplet reactors fit into a millilitre tube and an equivalent number of experiments can be carried out simultaneously. The key idea is that the droplet compartment combines the functional molecule with information on its identity and a readout of its function (Fig. 1). Thus the droplet contains everything needed to assess and decode a particular experiment or profile of a library member. Water-in-oil emulsion droplets

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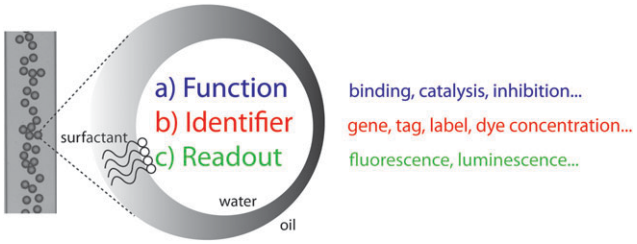

Fig. 1 The droplet compartment combines (a) the function of a molecule (*e.g.* the catalytic activity of an enzyme or the inhibitory function of a small molecule), (b) the information on its identity (*e.g.* its sequence encoded by DNA or an optical label) and (c) a readout to assess the molecule's ability to carry out its function *via* a miniaturised assay (*e.g.* based on product fluorescence). Droplet size varies between 1 and 200 μm in diameter corresponding to volumes between 0.5 fl and 4 nl.

can be easily made by mixing oil and water using a stirrer, homogeniser or extruder. Droplets generated in this way have proven successful in many applications.[2–5]

However, droplets produced with bulk emulsion techniques are not uniform in size.[1,7] Complications arise in experiments where a quantitative readout is required, as these protocols are insufficiently precise to yield monodisperse droplets, with uniform volumes that allow stringent definition of concentrations therein. Another limitation of bulk emulsion droplets is that multistep processing of droplets is difficult, although some strategies for reagent delivery such as nanodroplet fusion, uncaging of substrates and adding of hydrophobic substrates through the oil phase have been demonstrated.[2]

This is why microfluidic devices have recently been built, in which up to 10 000 highly monodisperse aqueous droplets per second (typically 10–200 μm in diameter corresponding to volumes between 0.5 pl and 4 nl) are generated in a continuous oil phase. Biocompatible surfactant–oil formulations have been developed that prevent droplet coalescence, allow oxygen diffusion and prevent molecules leaking out into the oil phase.[8–13] In addition to droplet formation, the microfluidic format allows a number of other unit operations that are summarised in Fig. 2. Droplets can be divided, fused, incubated, analysed, sorted and broken up. Integration of these steps with control over timing can potentially create a system for biological experimentation with a level of control akin to experiments on the macroscopic scale.[14–17]‡

The choice of microfluidics for droplet 'management' also allows access to typical advantageous engineering features of this format, *e.g.* the potential for automatisation, the low cost of microfluidic devices and improved heat and mass transfer due to high surface area to volume ratios. In contrast to non-compartmentalised microfluidic systems, the droplet-based approach has the further advantages of rapid mixing of reagents (by chaotic advection) on the ms scale,[18,19] the lack of dispersion,[18] reduced interactions of reagents with channel walls and little or no cross-contamination between different compartmentalised samples. Most importantly, compartmentalised microfluidics allows easy parallelisation of independent experiments without increasing device complexity and size, thus achieving very high throughput.[15]

Conventional high-throughput technologies such as robotic microtiter plate platforms can reach a throughput of around 1 Hz, involving volumes as small as 1 microlitre per well.[20] Droplet-based microfluidic techniques are therefore up to four orders of magnitude superior in throughput (up to 10 kHz) and up to six orders of magnitude in reagent consumption compared to conventional high-throughput technologies: microfluidic droplets range between pico- to nanolitres and the smaller bulk emulsion droplets reach even into the femtolitre range. This extreme miniaturisation has the potential to reduce reagent consumption and thus costs per assay.

Recent reviews have focused on the operations that have been developed to manipulate microfluidic droplets and chemical reactions that can be performed in droplets.[14–17,21,22] Here we aim to provide an overview of biological experiments that have been performed in microfluidic droplets and outline the potential of these microfluidic platforms in future biological high-throughput experiments.

Droplets provide monoclonality

Commercially, the most successful application of compartmentalisation is the emulsion polymerase chain reaction (ePCR). The benefits of compartmentalisation for PCR go beyond an increase in throughput. ePCR enables clonal amplification of templates from complex mixtures in a bias-free manner, thus enabling a number of applications, most importantly high-throughput sequencing.

For ePCR the DNA molecules are segregated in individual droplets, such that each droplet contains no more than a single template, *i.e.* the droplets are monoclonal. Each template is amplified in isolation avoiding competition between multiple amplicons. Isolation of individual PCR reactions has been shown to prevent preferential amplification of one template over another due to differences in amplification efficiencies caused for example by different lengths and G/C contents. Amplification in isolation also prevents generation of artifactual fragments by recombination between homologous regions of different DNA templates. Thus, amplification by ePCR reflects the original composition of complex mixtures of genes, such as those encountered in genomic and cDNA libraries, much better than conventional PCR.[23]

Furthermore, compartmentalisation increases the effective concentration of the template in the droplet, permitting efficient single-molecule (reverse-transcription) PCR.[24–26] Compartmentalised PCR has been used for amplifying and linking two amplicons of two polymorphic sites on a single DNA template, thus enabling haplotyping.[27]

To recover the amplified DNA the droplets must be broken, potentially losing the monoclonality by pooling the content of all droplets together. This is not acceptable for applications in which the monoclonal nature of the product is essential, *e.g.* in sequencing. For these cases, monoclonality is maintained by capturing the DNA on a solid support, such as a microbead.

‡ Droplets can also be manipulated on open planar surfaces and moved by electrowetting, dielectrophoresis, or magnetic methods. These droplets that are not dispersed in an oil phase have been reviewed elsewhere.[17,173,174] Droplet handling in this format does not readily avail itself to high-throughput, so these methods are mentioned here only in passing.

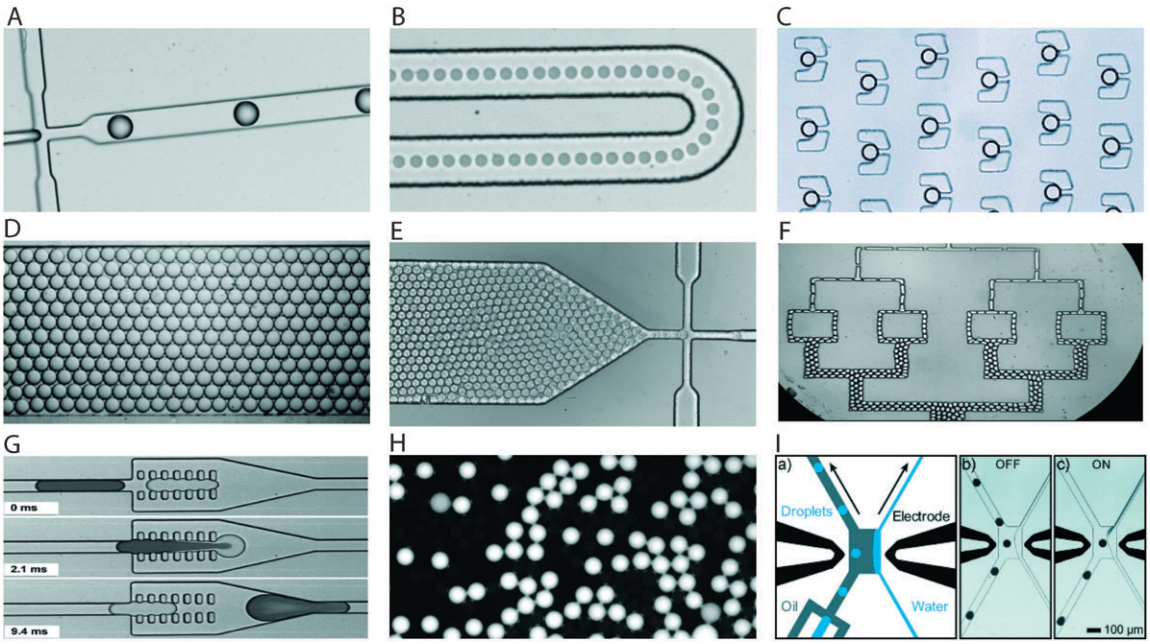

Fig. 2 Typical unit operations involving droplets in microfluidic devices. After the droplets have been formed (A) they can be kept moving in delay channels (B) or parked in traps (C) or reservoirs (D). The possibility exists to incubate the droplets offline and to re-inject them into the device (E) for further manipulations such as splitting (F) or fusion (G). The most frequently used readout is fluorescence (H). Fluorescent droplets can be sorted from non-fluorescent droplets. The selected droplets can also be directly broken and fused to a continuous flow of an aqueous phase (I). Acknowlegements: (C) From ref. 36. Reproduced by permission of the Royal Society of Chemistry. (D) From ref. 12. Reproduced by permission of the Royal Society of Chemistry. (F) Reprinted with permission from ref. 37. Copyright 2004 by the American Physical Society. (G) Reproduced with permission from ref. 38. Reproduced by permission of the Royal Society of Chemistry. (H) Reprinted with permission from ref. 11. (I) From ref. 39. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

If, in addition to DNA and the PCR mixture, the droplets contain a microbead on which one of the two primers is immobilised, the amplified DNA becomes attached to the solid support (Fig. 3).[28] These monoclonal beads, carrying several thousand copies of the single DNA molecule originally present in the droplet, can be assessed by fluorescent labelling and counted by flow cytometry. Although the efficient amplification on beads is limited to relatively short amplicons (<250 bp),[28] detection and quantification of rare genetic variations[29,30] and high-throughput screening of transcription-factor targets[31] are possible. ePCR on microbeads is a key step for the high-throughput second generation sequencing platforms 454,[32] SOLiD[33] and Polonator[33] where *in vitro* clonal arrays are sequenced, avoiding the potential bias and the bottlenecks of transformation and colony picking that bacterial cloning introduces.[34,35]

For these applications the droplet maintains monoclonality and removes the bias due to competition of different templates during PCR. These requirements are met by polydisperse bulk emulsion droplets. However, the use of monodisperse microfluidic droplets has the potential to extend the usefulness of existing emulsion PCR protocols: the application of microfluidics enables integration of PCR with other droplet operations such as fusion and real-time analysis and uniform droplet sizes allow a quantitative analysis, as required for quantitative real-time PCR.

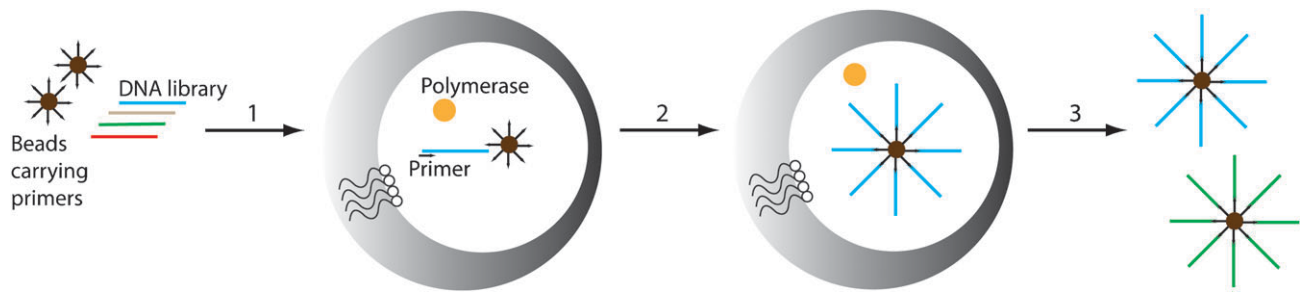

Fig. 3 Schematic of emulsion PCR (ePCR) on microbeads. Beads coated with primers and a DNA library are dispersed into droplets together with the second free primer and all necessary components for PCR (1). The droplets are thermally cycled as in conventional PCR. The amplified templates become attached to the bead through the bead-bound primer (2). The emulsion is broken, but only one type of DNA molecule is attached to one bead preserving the monoclonal nature of the amplified material. The beads can now be used for further analysis such as sequencing and haplotyping. Figure adapted from ref. 29.

A specific example is an approach to targeted sequencing developed by RainDance Technologies—the first commercially available platform using microfluidic droplets.[40,41] Their platform will enable enrichment for targeted (re-)sequencing for numerous biomedical applications, including genomics research, gene expression analysis, drug development, and key marker detection of diseases for personalised medicine. For the latter application a collection of primer pairs corresponding to selected genomic regions are encapsulated in microfluidic droplets and then merged with droplets containing the genomic DNA and the PCR reaction mixture, followed by off-chip thermocycling.[174] The amplification products of enriched sequences are recovered by breaking the emulsion, purified and processed for second generation sequencing.[32,33,42,43] Here, microfluidics enable controlled droplet fusion, allowing individual combination of members of a primer library with the genomic DNA. In turn, the use of monodisperse droplets improves target enrichment uniformity, reducing the amount of oversampling necessary for the reliable detection of rare alleles and thus saving sequencer capacity. Similarly, Kumaresan and colleagues have used a microfluidic droplet generator and incubation in a benchtop PCR machine to perform emulsion PCR of fragments up to 1139 bp on microbeads from single DNA molecules or cells for sequencing and genetic analysis.[44]

More recently, ePCR has become a module that can be readily integrated into microfluidic devices, thus allowing direct integration with other droplet unit operations such as fluorescence-based monitoring of the amplification. To this end the entire device can be thermocycled,[45,46] but devices with inbuilt temperature profiles and a continuous droplet passage have improved throughput. Amplification in a microfluidic device was monitored online by recording the fluorescence of a Taqman-based FRET probe for the amplicon.[45–47] The agreement between the observed number of droplets in which amplification has occurred and the predicted number of droplets containing a DNA template according to Poisson statistics showed the feasibility of 'digital PCR' in microfluidic droplets. 'Digital PCR' is a method to detect and quantify minute amounts of DNA, *e.g.* in medical diagnostics or in analytical applications.[48–50] The analysis of a 'digital PCR' experiment is based on a count of the total number of droplets and droplets in which amplification was successful (corresponding to the presence of template DNA in these droplets). Thus 'digital PCR' transforms exponential analogous data from conventional PCR to more reliable linear digital signals.[48–50]

The addition of a reverse-transcription step also opens the door for gene expression profiling and the detection of viral RNA.[45] For digital PCR a fluorescent endpoint measurement is sufficient, so the droplets do not have to be watched continuously. The limit of detection is defined by the number of compartments,[48] so high-throughput is important. To improve the throughput of microfluidic droplets PCR continuous-flow has been employed.[51–58] In continuous-flow PCR the reaction mixture passes through zones of alternating temperature corresponding to denaturation, annealing and extension temperatures. This format avoids temperature cycling of the entire device and leads to more rapid heat transfer and faster throughput than batch PCR microfluidic

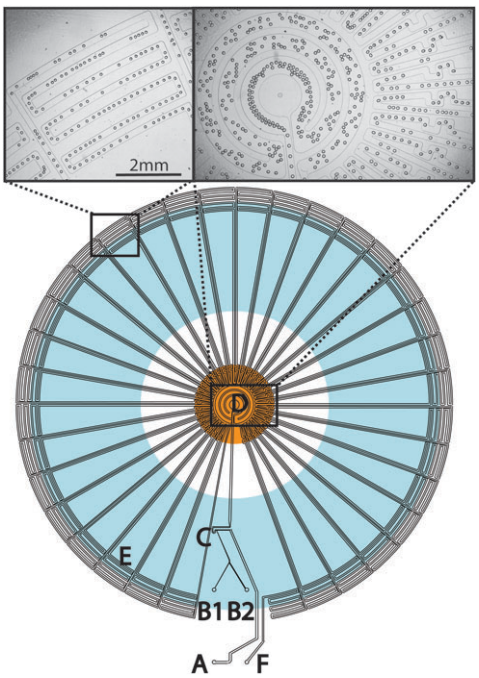

Fig. 4 A PCR device in which microfluidic droplets move through a temperature gradient across the radial design. The device contains an oil inlet (A) that joins two aqueous inlet channels (B1 and B2) to form droplets (C). The droplets pass through the inner circles in the hot zone (D) to ensure initial denaturation of the template and travel on to the periphery where primer annealing and template extension occur (E). The droplets then flow back to the centre, where the DNA is denatured and a new cycle begins. Finally, the droplets exit the device after 34 cycles (F). The positions of the underlying copper rod and the Peltier module for temperature control are indicated with orange and blue areas, respectively. Figure reprinted with permission from ref. 51.

chambers. Smaller droplets allow higher throughput so the scale-down from millilitre[52,53,56,57] to nano- to picolitre droplets in continuous-flow microfluidic PCR (Fig. 4) may lead to higher sensitivity.[51,54,55]

PCR in these formats matches its macroscopic equivalent: amplification is specific, has comparable efficiency[51] and can be reliably quantified.[54] Droplet-based continuous-flow PCR also presents an alternative to non-compartmentalised microfluidic PCR devices,[59] in which interactions of channel walls with polymerases and template DNA limit the biocompatibility of such systems and lead to cross-contaminations.[60,61]

Although PCR is the most common DNA amplification strategy, it is not the only one. Several isothermal DNA amplification methods exist, which do not require thermocycling.[62] This makes integration with other droplet operations and biochemical experiments convenient, since no equipment for thermal cycling of the droplets is necessary. Mazutis *et al.* showed that digital quantification of DNA in microfluidic droplets is also possible using random primers and the DNA polymerase from bacteriophage phi29 in an isothermal reaction.[63] This hyperbranched rolling circle amplification (HRCA) or multiple displacement amplification (MDA) can also yield sufficient DNA for sequencing of single cells.[64] It has been shown that reducing the reaction volumes to nanolitres reduces non-specific synthesis and amplification bias.[65]

Droplets link pheno- and genotype

In vitro compartmentalisation (IVC) was initially developed for *in vitro* directed evolution. Here the droplet boundary serves as the equivalent of the cell wall to link the genotype (DNA or RNA) to the phenotype (an observable trait, such as binding or catalytic activity).[1] This phenotype–genotype linkage is essential to mimic natural selections, in which cells compartmentalise genes and proteins, to create proteins or nucleic acids with improved or new functions.[66] For such a molecular evolution experiment, droplets are formed that contain no more than a single member of a nucleic acid library. The genes are transcribed and translated by an *in vitro* transcription/translation (IVTT) extract derived from *Escherichia coli*, wheat germ or rabbit reticulocyte lysates. Droplets containing a desired phenotype are selected by a suitable strategy. This approach has the advantages of being a complete *in vitro* system: expression of proteins that are toxic to host cells and incorporation of non-natural amino acids are possible, no restrictions of transformation efficiency apply (typically limiting the library size to approximately 10^8–10^9), the selection environment is not limited to conditions compatible with cell survival (such as pH, temperature or co-solvents)[66] and the selection pressure cannot be circumvented through mechanisms not directly related to the function of interest, as can be the case with *in vivo* evolution.

Many contemporary directed evolution experiments use cells to provide the link between phenotype and genotype. Cells contain more than just one copy of a plasmid and therefore the protein yield can be higher than with IVTT. Cells can also provide additional co-factors for folding, post-translational modifications or activity of proteins. However, cell colony assays on agar plates are only end point assays and depend upon a precipitating product. The utility of FACS assays is also limited to fluorophores that remain in or on the surface of cells.[67] In contrast, if cells are compartmentalised in droplets any protein or product released by the cells remains contained in the droplet. The phenotype–genotype linkage is ensured and the droplet contained can be analysed, for example with fluorescence microscopy.

Bulk emulsion droplets have been applied to evolve catalytic properties of enzymes and RNAs, binding of peptides and proteins as well as regulatory activities. Directed evolution experiments in bulk emulsion droplets have been reviewed in detail elsewhere.[2–5] Therefore we limit the discussion here to representative examples illustrating the principles (Fig. 5).

The selection strategies pursued can be grouped into three main categories:

(A) The genotype (nucleic acid) is not only the carrier of the genetic information but is also the substrate. The desired function to be evolved alters the nucleic acid in such a way that it can be easily separated from unmodified substrates coding for non-active proteins. An example is the evolution of polymerases with new properties such as increased thermo-stability or an altered substrate range.[68–72] The polymerases and their genes are subjected to PCR in the droplets. This compartmentalised self-replication (CSR) confers a selective advantage on active mutants because its genes are amplified and have a greater chance of being recovered and passed on to the next round of evolution. CSR uses *E. coli* cells rather than an IVTT system to express the polymerase.

(B) The second selection strategy is based on sorting of fluorescent droplets or beads. Any product produced by the encapsulated enzymes or cells also remains compartmentalised within the droplet and is therefore linked to the genotype. If a non-fluorescent substrate is converted into a fluorescent product, droplets containing an active catalyst can be distinguished from non-fluorescent droplets. This can be done directly on chip for microfluidic droplets, but an additional emulsification step is required for bulk emulsion droplets. The resulting double emulsion droplets can be sorted by FACS.[73–75]

Microbead display is another strategy that takes advantage of sorting by fluorescence.[76–79] Beads carrying one gene of a library, each with an epitope tag, and antibodies against this tag are compartmentalised in droplets with IVTT. The translated proteins become attached to the beads *via* the epitope tag–antibody interaction. The emulsion is broken and the beads, displaying multiple copies of the protein, are isolated. To select for binding by FACS the beads are incubated with ligands coupled to a reporter. Given that the number of proteins immobilised per bead with a single DNA template is maximally 300 in small bulk emulsion droplets (around 3 μm in diameter) a reporter is required to amplify the signal.[77] Therefore Gan and colleagues[79] first amplified the DNA on the bead using emulsion PCR. Together with the use of bigger droplets (around 30 μm) enough protein was immobilised to sort the incubated beads directly with a fluorescently labelled ligand by FACS. Microbead display was also used to select for enzymatic activity.[76] The advantage of microbead display is that the reaction conditions do not have to be compatible with IVTT, since an enzyme can be displayed, purified and re-emulsified in different conditions without losing the genotype–phenotype linkage. This method is useful, if for example the selected activity is present at a high background level in the IVTT mixture.

(C) In the third selection category a stable DNA–protein linkage is formed in the droplet which persists after the emulsion is broken. In affinity selections for binding by 'panning' against a target molecule, binders are enriched and can be decoded *via* the attached DNA. The link between protein and DNA can be made in a number of ways.

Microbead display has already been described above, but affinity selections of the displayed protein against immobilised ligands on a solid phase are impossible, as the weight of the bead precludes panning.[77,78] In STABLE,[80] the expressed proteins are fused to streptavidin and become non-covalently attached to its biotinylated coding DNA. In M.Hae III display,[81] the conjugation is covalent and occurs *via* a DNA-methyltransferase (M.Hae III) fused to the protein of interest, which reacts irreversibly with a fluorocytidine analogue present at one end of the coding DNA fragment. Likewise, a covalent linkage is formed in the SNAP-display developed by Stein and co-workers,[82] where a SNAP-tag (O^6-alkylguanine-DNA alkyltransferase) reacts covalently with its suicide-substrate O^6-benzylguanine (BG) incorporated into the linear coding DNA templates. The latter systems with covalent linkages should allow selections under distinctly

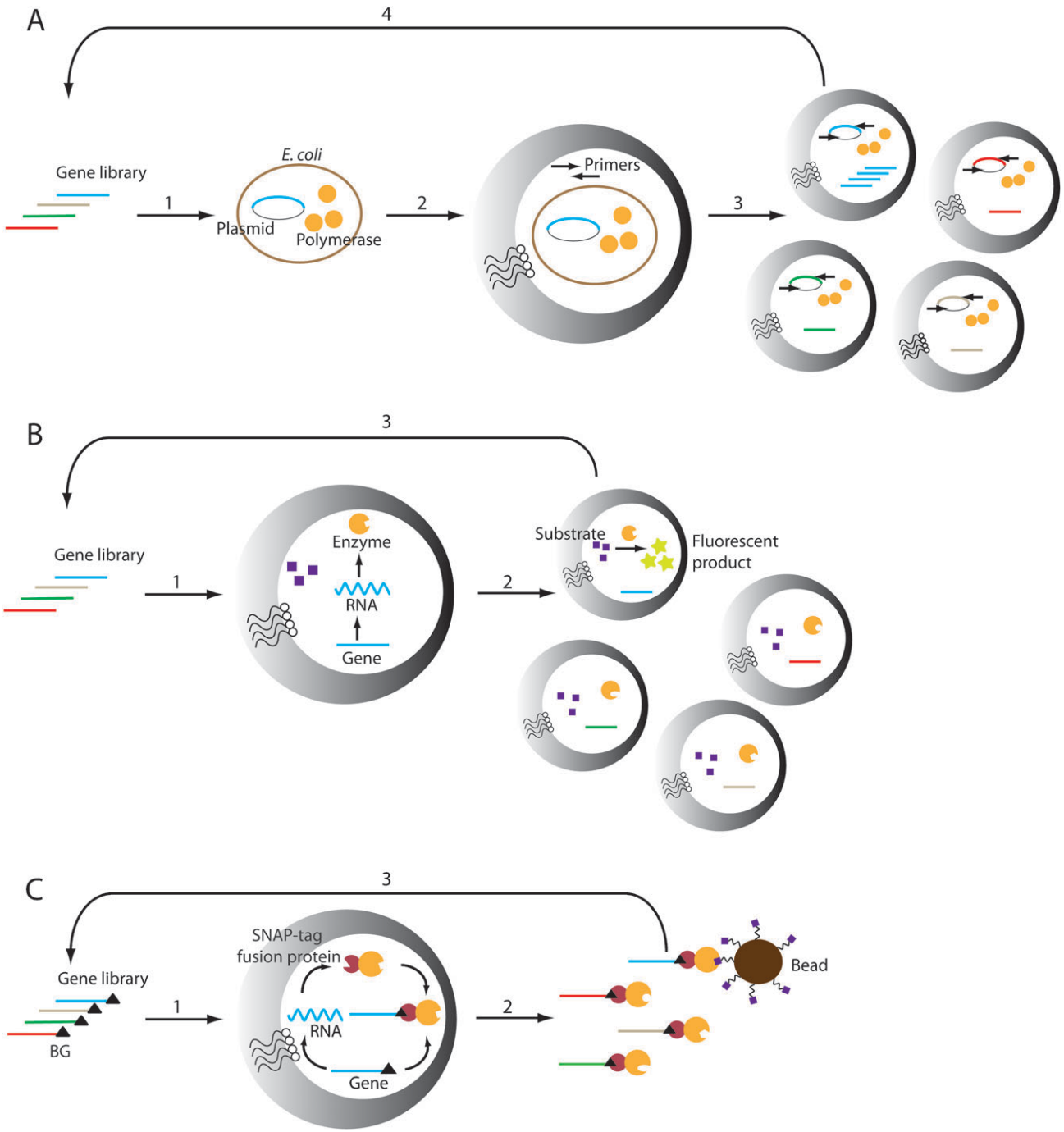

Fig. 5 Schematic of exemplary directed evolution experiments performed in water-in-oil droplets. (A) Directed evolution of polymerases by compartmentalised self-replication (CSR). A library of genes are cloned and expressed in *E. coli* (1). The cells are dispersed into droplets together with primers and dNTPs (2). The droplets are subjected to thermal cycling. The polymerase and its gene are released from the cell, allowing self-replication by PCR (3). Successfully amplified genes are recloned for further rounds of selection (4). Figure adapted from ref. 68. (B) Directed evolution of enzymes using a fluorescence-dependent sorting. A library of genes is dispersed into droplets (1). The genes are *in vitro* transcribed and translated. Active enzymes convert a non-fluorescent substrate into a fluorescent product (2). Fluorescent droplets are separated from non-fluorescent droplets. If a fluorescence-activated cell sorter (FACS) is used to do so, the water-in-oil emulsion has first to be converted into a water-in-oil-in-water emulsion (double emulsion). In microfluidic devices the droplets can be sorted directly. Genes in sorted droplets can be amplified by PCR and used for further rounds of selection (3). Figure adapted from ref. 74. (C) SNAP-display: a covalent display system for directed evolution of protein binders. A library of genes fused to a SNAP-tag (O^6-alkylguanine-DNA alkyltransferase) is dispersed into droplets. The DNA is labelled with BG (O^6-benzylguanine) (1). The genes are *in vitro* transcribed and translated. The SNAP-tag fusion protein becomes covalently linked to its own coding DNA. The emulsion is broken, and protein binders bind to immobilised binding partner. (2) Bound genes can be amplified by PCR and used for further rounds of selection (3). Figure adapted from ref. 82.

non-natural conditions. In addition, displayed proteins can be chemically modified using methods[83,84] that would be incompatible with non-covalent *in vitro* display systems, such as ribosome display,[85] or with biological display systems such as yeast[86] or phage display.[87]

Given the impressive array of functional modules in microfluidics (Fig. 2), it is perhaps surprising that the principles that appear to work in emulsions made by bulk protocols have not yet been applied to directed evolution experiments in microfluidic droplets. The challenge here is integration of the physical droplet processing steps with standard biological operations that may later be part of an integrated device for directed evolution. The following biological experiments in microfluidic droplets suggest that directed evolution in this format will eventually become a reality. First, compartmentalisation of cells is possible: single bacteria or yeast cells can be cultivated in droplets and recovered alive.[11–13,36,44,88–102] Second, *in vitro* protein expression has been demonstrated in microfluidic droplets.[10,12,63,103] The successful protein expression was quantified by measuring a fluorescent protein (GFP)[10,103] or the turnover of non-fluorescent fluorescein di-β-D-galactopyranoside into fluorescein.[12,63] Courtois *et al.*[10] observed high expression yields for GFP (up to 30 000 molecules per DNA template). These high yields made it possible to perform protein expression from single copies of the plasmid DNA—monoclonality being a pre-requisite for directed evolution experiments. Also, kinetic parameters for several enzymes[9,19,36,104–109] were determined in microfluidic droplets, providing the facility to evaluate individual mutants kinetically. Due to rapid mixing in droplets an accurate description of reaction kinetics is possible, even at the millisecond scale.[18,19,109] The enzyme activity of (over-)expressed proteins in cells can be measured if the protein is exported,[90] the cells are hydrolysed in the droplet[97] or the substrate can be taken up by the cell.[75] Baret *et al.* demonstrated the fluorescent activating sorting of droplet containing *E. coli* cells, expressing either the reporter enzyme β-galactosidase or an inactive mutant.[102]

Further integration of physical and biological unit operations and modules must be achieved to build a real 'directed evolution machine'. The running of a highly integrated device is certainly more difficult than that of present models. An alternative, at least temporary, solution is to uncouple modules and use a different device for each function. The droplets can be transferred from one device to the other,[63,98,102,110] provided the surfactant is able to stabilise droplets sufficiently in transit.[12,13]

Initially libraries will not contain very active hits, so detection of mutants with low activities will be difficult or impossible. Droplets should just contain one gene copy, maintaining monoclonality of the droplets, but the yield of protein expression from single genes is limited. The successful examples of IVTT in microfluidic droplets might encompass only favourable cases. For example, plasmid templates, rather than PCR-generated DNA, were used. Amplicons generated by PCR are the standard templates for *in vitro* selection systems, but give generally lower yields in IVTT systems because of their lack of plasmid supercoiling[111] and their sensitivity to nucleases. So how will proteins with inefficient *in vitro* expression be detected? A DNA amplification step in which the amount of DNA—and later RNA and protein—is increased might then become crucial, broadening the dynamic range of detection downwards and reducing the loss of diversity in the beginning of the selection process. Indeed, isothermal DNA amplification has been shown to increase the amount of *in vitro* expressed β-galactosidase in droplets initially containing one gene copy.[63]

Encapsulation of particles and molecules into droplets follows the Poisson distribution. In order to obtain mainly monoclonal compartments, most of the droplets will be empty. For example, a suspension containing on average 0.3 DNA molecules per droplet results in 74%, 22% and 3% of the droplets containing none, one, or two molecules, respectively. The problem is amplified when two types of particles (*e.g.* beads and DNA) are compartmentalised in droplets carrying one particle of each kind.

Currently, the throughput achieved for droplet formation in a single microfluidic device is smaller than when droplets are generated in bulk emulsion. At a rate of 10 kHz it takes 11.5 days to generate 10^{10} droplets with a device compared to 5 minutes in bulk. A scale-up is, however, possible. For example, Nisisako and Torii developed a device with up to 256 droplet-formation units for mass production of monodisperse droplets[112] and Damean *et al.* built a device with four strings of droplets for simultaneous monitoring.[104] It is not always necessary to have huge libraries, often targeted and designed libraries yield adequate results.[113] Neutrally drifting a library (*i.e.* gradually accumulating mutations under selection for the protein's original function) prior to selection for a new function has been shown to increase the likelihood of identifying hits, reducing the library size necessary to find a functional solution.[114]

Despite lower throughput in microfluidics advantages over bulk emulsion experiments are expected. Precise manipulation and control promise to allow access to otherwise impossible experiments. For example, droplet contents can be PCR-amplified and combined with the IVTT mixture. Alternatively, droplets containing an IVTT mixture can be fused to droplets with a labile substrate, allowing temporal separation of protein expression and activity assay. The high monodispersity of microfluidic droplets enables performance of quantitative assays that are impossible in the polydisperse bulk emulsion droplets. The measuring of quantitative kinetic properties of an entire collection of mutants will enable comparison of fitness landscapes[115] of different libraries. There are indications that droplet formation in microfluidics is gentler and inactivates proteins less than the rather vigorous methods for formation of bulk emulsion droplets.[10] The microfluidic droplets are generally bigger (10–200 μm in diameter) compared to bulk emulsion droplets (1–10 μm in diameter). Larger volumes can, in certain circumstances, be the better choice because they can contain more reagents. For example, the number of displayed proteins on a bead for the microbead display should be significantly improved from 200 to 300 per bead[77] in bulk emulsion droplets to several thousand copies in microfluidic droplets, enabling direct fluorescent detection without a signal-amplifying cascade. The online detection of fluorescence and subsequent sorting of positive droplets (up to 2 kHz)[102]

can substitute for the formation of double emulsion droplets and their fluorescence-activated sorting (FACS, up to 10 kHz).

Screening of metagenomic libraries

An alternative to directed evolution of new enzyme activities is a functional search for novel genes in metagenomic libraries, which have proven a rich source of novel biocatalysts for biotechnological and pharmaceutical applications.[116–118] Such libraries are constructed from isolated microbial samples derived from environmental DNA. They often originate from organisms that are either unknown or cannot be cultivated and thus have to be expressed in a host strain. Heterologous expression results in low success rates due to the lack of efficient transcription, translation, folding or secretion of the metagenomic genes.[118] The ensuing problem of low screening success rates (screening of several hundred thousand clones to find only a few active clones[118]) is usually addressed by large-scale automatisation and miniaturisation. Performance of these screens in droplets promises to reduce the time and cost of this effort. The challenges highlighted in the discussion of directed evolution equally apply for screening of metagenomic libraries. However, to date there is no published example of this approach in bulk or microfluidic droplets.

Droplets as an economical format in high-throughput screening: drug discovery

The process of drug discovery deals with smaller libraries than directed evolution, but the cost of the reagents involved—cells, proteins and small molecules—is substantial and contributes to the cost of screening ($\sim$1$ per assay).[119] Miniaturisation of assay reactions in microfluidic droplets could reduce this cost by at least a factor of 1000 compared to microtiter plate platforms, corresponding to the reduction in assay volume.

In principle, assays used in pharmaceutical research are no different from those used in directed evolution. Enzyme kinetics or cell-based assays could be miniaturised. Generating a concentration gradient[104] is the basis for obtaining *e.g.* inhibition curves where a measurement of the K_i or IC_{50} gives access to structure–activity relationships. This principle has been illustrated by Brouzes *et al.* in a determination of an IC_{50} for the chemotherapeutic drug mitomycin C on a human monocytic cell line (U937).[110]

Experiments in directed evolution rely on DNA for decoding successful hits. Small molecules lack the ability to be amplified and sequenced, so alternative decoding approaches have to be explored. Several strategies are conceivable, but none of them have yet been demonstrated in droplets. First, successful hits can be analysed directly, for example by mass spectrometry, which can be coupled to microfluidic devices and allow decoding of droplet contents.[120–122] A second strategy is to co-compartmentalise or attach the library members to DNA fragments,[123] quantum dots[124] or colloidal support beads containing an optical signature,[125] which act as bar codes that can be decoded. A physical link between the chemical compound and the bar code carrier is not necessary, as long as both are compartmentalised in the same droplet. The stability of emulsion formulations would allow a format in which a small molecule library member is labelled and compartmentalised just once and remains available for future use within a droplet, which is fused with an assay reaction droplet when desired. This compartmentalisation can take place immediately after compound synthesis or can rely on current liquid handling systems that are able to transfer libraries from a multi-well storage format into its compartmentalised equivalent.[110] Edgar *et al.* integrated the spatial confinement of compounds into droplets after their separation with capillary electrophoresis.[126] This concept might also be applied to other separation techniques such as HPLC.

In addition to the identity of the potential inhibitor, knowledge of its concentration is desirable. Methods where the decoding can happen in real-time are convenient. One possibility is to add a fluorescent dye with variable concentration as a fluorescence code in order to mark the concentration of the analyte.[108,110,127] Different dyes can be used for different solutions. The disadvantage of this method is possible contamination or interference of the fluorescent dye with the assay, especially if the assay readout is also fluorescence, although different fluorescence channels can be used. Alternatively, droplet pairs have been used, in which the first droplet contains the reaction mixture and the second is used to index the composition of the first. Intensity ratios of dyes in the second droplet indicate the ratio of reagents used in the first droplet.[128]

For structure-based drug discovery, protein crystallisation is often the rate-limiting step in determining the structure of proteins by X-ray crystallography.[129] However, reliable guidelines for the generation of crystals are lacking. Instead, trial-and-error is necessary to identify experimental conditions that support crystallisation, by screening many different compositions of crystallisation solutions. At the same time proteins are often only available in limited amounts. Therefore, different strategies for the miniaturisation of the crystallisation process have been pursued, including the use of microfluidic droplets. Droplets have been extensively and successfully used in a high-throughput manner to screen for, optimise and perform protein crystallisation as well as to improve the understanding of its fundamentals. Protein crystallisation in microfluidic droplets has already been reviewed, so we refer the reader to this detailed body of literature.[14,15,130,131] As for drug discovery, a significant challenge is the indexing of droplets by their content. Indeed, some of the indexing strategies mentioned above[127,128] were initially demonstrated for labelling crystallisation trial droplets.

Droplets compartmentalise cells

Compartmentalisation of an increasing number of cell types—*e.g.* bacteria,[11,36,44,88–92,102,132] yeast[12,93,94] and mammalian[13,44,96–101,108,110,133] cells (Fig. 6A)—is possible. Cells have been shown to remain viable in several oil–surfactant mixtures and device designs. While it is possible to keep the droplets flowing in serpentine channels for several minutes,[107] long-term storage requires droplets to be stationary, *e.g.* in special droplet spots,[94] traps[36] or reservoirs.[11] It is also possible to incubate the droplets up to several days offline and re-inject into a device for analysis.[13,110] Fluorinated oil

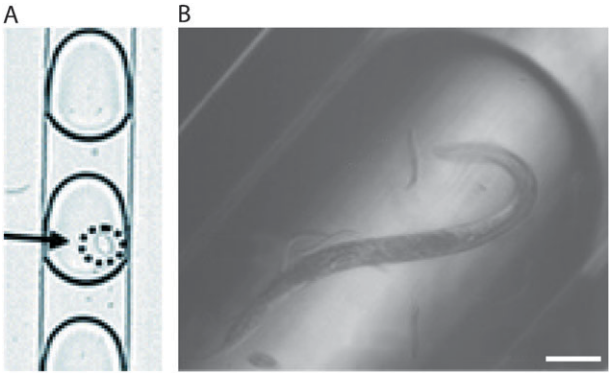

Fig. 6 Encapsulation of cells and organism in microfluidic droplets. (A) A mammalian CHO-K1 cell in a 65 pl droplet (approximate diameter: 50 μm). From ref. 133. Reproduced by permission of the Royal Society of Chemistry. (B) Nematode *C. elegans* 660 nl droplet together with its larvae. These latter droplets far exceed the usual size of droplets discussed in this review. White bar, 100 μm. Figure reprinted from ref. 13 with permission from Elsevier.

phases seem to be particularly well suited for long-term storage of cells, on account of their oxygen permeability.[12,13,110] However, for experiments on the timescale of hours to a day,[11] the oxygen adsorbed in normal oil suffices. For cells heavier than *E. coli*, mechanisms have been developed to circumvent the limits of Poisson distribution, *i.e.* maintain single-cell occupancy avoiding a large fraction of empty droplets.[95,96]

With dilution on chip, *i.e.* varying the ratio of the flow rates between the aqueous solutions before the droplets are formed, it is easy to produce droplets containing different concentrations of a molecule of interest. However, studies applying these techniques for cell-based assays are only now emerging. For example, Boedicker and colleagues tested the sensitivity of bacterial strains to different antibiotics and determined their minimum inhibitory concentration.[88]

In contrast to flow cytometry,[134] much greater control over the environmental conditions in a droplet is possible, and secreted as well as intracellular components cannot escape the droplet compartment. The small compartment size creates a high local concentration and this leads to high sensitivity of detection. In addition, time courses rather than spot-checks can be carried out and, in principle, cells in droplets can be interrogated by multiple optical methods. Molecular markers for cellular processes include fluorescent proteins or reporter enzymes[135,136] and imaging of cell morphology for cell biological analysis.[137,138] These methods allow the simultaneous determination of multiple characteristics and their time-dependent changes. If reagents can be delivered by fusion modules with rapid mixing, this platform should be ideally suited for 'high-content screening',[134] where multiple lines of evidence are adduced to record a more complex picture, *e.g.* the effects of a small molecule modulator or other external stimuli.

Single-cell studies are not only highly economical, which could be important for precious cell lines and reagents, but also allow access to information that is unavailable in conventional experiments. In particular, it will be interesting to study mechanisms that control cellular responses to environmental conditions or other external stimuli. The response of a cell population to the signal may appear linear, but at the level of a single cell responses are often governed by an all-or-nothing principle. Below a certain signal threshold, the response is off, but as the threshold is surpassed a rapid response occurs.[139] The group of Xie has shown that it is possible to monitor stochastic protein expression in single *E. coli* cells in single phase microfluidic chambers.[140–142] Microfluidic droplets seem to be an ideal tool for the better understanding of such stochastic molecular mechanisms.

Kinetics of enzymes expressed by cells have been recorded at second to minute[90] as well as minute to hour time scales.[94] Huebner and colleagues detected the overexpression of yellow fluorescent protein in *E. coli*,[91] whereas He *et al.* measured the activity of endogenous β-galactosidase of mouse mast cells after lysis inside droplets by a laser.[97] When a catalytic protein, alkaline phosphatase, was co-expressed with red fluorescent protein (RFP), the expression level of the marker could be used to normalise the activity observed. As simultaneous determination of catalytic activity and expression level was monitored at the level of single cells, the dynamics of expression and catalysis in individual members of a population become accessible.[132] Hufnagel and colleagues immobilised, cultured and transfected mammalian (CHO-K1) cells with a GFP plasmid on chip under non-compartmentalised conditions. Afterwards the cell suspension was directly transferred into a droplet-making device for subsequent single-cell analysis, thus integrating microfluidic multistep cell culture manipulations and analysis in droplets.[133] Other cell manipulation tools such as electroporation,[93,101] freezing[99] of cells and sizing of subcellular organelles[143] have also been implemented in microfluidic droplets.

Live–dead assays[13,98,110] suggest that the cells survive in the droplets, but hardly grow and it remains to be seen whether the behaviour of the cells in droplets reflects the behaviour in cell culture, especially for adherent cells. A possible solution is encapsulation in a hydrogel, such as alginate, to create 3D cultures.[100] A different approach is the 'chemistrode'—a system for cell stimulation and analysis. Cells rest on a glass surface and droplets are directed *via* microchannel towards them to deliver stimuli. Response molecules secreted by the cells are likewise carried away by droplets for online and/or offline analysis.[144] This methodology was demonstrated for stimulation of a murine islet of Langerhans (cluster of hormone-producing cells of the pancreas) with increasing glucose concentrations and subsequent measurement of insulin secretion at a 0.67 Hz frequency.

Even organisms as large as nematodes (*Caenorhabditis elegans*) have been successfully compartmentalised in, albeit unusually large (660 nl), droplets (Fig. 6B).[13] The worms hatched from their eggs, grew to adults and laid new eggs within the droplets. In such large droplets it is also possible to encapsulate *Drosophila melanogaster* embryos[145]—another model organism for studies in developmental biology.

Droplets to probe protein–protein interactions

Understanding the functions of proteins lags far behind DNA sequencing of genomes. *In vitro* technologies for effective

analysis of enzyme–substrate reactions, protein–protein interactions and protein modifications could help to address this imbalance. Thus, proteomic studies stand to benefit from high-throughput protein expression and screening. Recently, Goshima *et al.*[146] presented a human open reading frame (ORF) collection of 33 275 clones and their *in vitro* expression. In droplets this human ORF collection could be expressed *in vitro* and screened in a high-throughput manner conducting any available assay. Porter and colleagues reported a cell-free approach for the interrogation of protein–protein, protein–DNA and protein–RNA interactions and their antagonists using a split-protein reporter.[147] Such protein complementation systems[148,149] have frequently been used in cells to detect protein–protein interactions and conducting this experiment in droplets provides its *in vitro* equivalent. The reporter enzyme firefly luciferase was split into two parts, each fused to another protein and only regained its activity when the two parts were brought together due to interaction of the two fused proteins with each other. Individual protein–protein interactions can also be detected directly, without a protein reporter: Srisa-Art *et al.* studied the binding kinetics of streptavidin and biotin using fluorescence resonance energy transfer (FRET) between two fluorescent dyes[150,151] as well as between angiogenin and an anti-angiogenin antibody.[152] The labelling of a whole proteome with such dyes in droplets is not feasible, but FRET can also be detected between fluorescent proteins that can be expressed as fusion proteins. Since mixing and detection in droplets are ultra-fast,[153] FRET systems could also be used to study protein folding and unfolding.[151]

Droplets for diagnostic assays

Microfluidic droplet platforms might well soon be present in specialised diagnostic laboratories. Digital PCR devices are ideally suited for routine medical diagnostics and microbial detection.[45,46,51,54] Other systems have been described for diagnostic tests. For example, low-abundance cell-surface biomarkers can be detected by enzymatic amplification in a droplet version of an ELISA assay.[108] Zhang *et al.* performed DNA methylation analysis using methylation-specific PCR in microfluidic droplets.[154] Song and colleagues developed a titration assay for an anticoagulant drug into blood samples and determined the clotting times using a microfluidic droplet device. The application of the correct dose of this drug is important, as too much can result in strong bleeding, whereas too little will not be effective.[155] Many cell-based assays could also be adapted for clinical purposes. For example, the hydrodynamic self-sorting of rare disease-causing bacterial cells present in a background of non-pathogenic bacterial cells has been demonstrated by Chabert and Viovy.[95] Another example is the detection of bacteria in a sample of human blood plasma.[88]

Droplets for biological engineering

The field of 'synthetic biology' comprises two main branches. One aims to create artificial life with unnatural molecules and the other to combine parts from nature into systems exhibiting unnatural functions.[156] Both areas share a key idea that biological processes can be engineered. This means that conventional engineering cycles of design, modelling, fabrication and quality control can be used to systematically steer the emergence of biological function. As in industrial process design, this relies on well-defined modules, which can be modified, repurposed and combined for the construction of new devices—and the devices then serve a role in manufacturing a biological system.[157]

The state-of-the-art techniques in microfluidic droplets described above seem ideally suited to the working logic of synthetic biology.[158] Fig. 2 summarises the modules for physical unit operations. The ability to create artificial compartments as evolutionary units, perform PCR, express proteins, compartmentalise bacterial and eukaryotic cells and analyse each compartment adds established biological unit operations. Together they provide a toolbox of a foundational technology. Now the challenge is to integrate modules, design circuits and create methods for steering modular iterative development cycles.

An example for the first branch of synthetic biology is *in vitro* evolution of polymerases[71,159] and ribosomes[160,161] using unnatural nucleotides and amino acids,[162,163] respectively. In more complex *in vitro* scenarios co-evolution of orthogonal DNA amplification and transcription/translation machineries might be possible.

The engineering of microbial consortia is an example for the second branch. Evolution of multiple interacting microbial populations can have advantages over evolution of one gene. By exploiting differentiation of function in synthetic consortia, results can be achieved that are not possible with individual populations.[164] Compartmentalisation of single bacterial species[11,36,44,88–92,132] in droplets and their fusion to create defined populations could create such consortia. The unit of evolution would thus be expanded compared to conventional evolution of single nucleic acids or proteins. Alternatively, cell–cell communication (*e.g.* quorum sensing)[165] could be studied[166] or evolved. The microfluidic droplet systems provide a high level of control, *e.g.* for varying consortia composition and environments. It also provides the possibility for optical detection, which would yield information on every droplet and screening in a high-throughput manner, in contrast to typical genetic selections from bulk mixtures of microbes. Such information would provide the basis for developing and validating models of complex systems of this sort, providing a tool for studying microbial interaction and evolution.

Challenges and future prospects

The feasibility and potential of systems involving microfluidic droplets are clearly demonstrated by the increasing number of experimental studies reviewed here—and there is no shortage of ideas for future applications. Specialised equipments such as fast cameras, high voltage suppliers, lasers and sensitive detectors, as well as a clean room facility for device fabrication, are currently necessary to carry out such experiments. However, commercial equipment for making droplets is becoming available, allowing access to this methodology by a broad biological community in basic biological and pharmaceutical research as well as in clinical laboratories.

It is, however, also clear that there is some way to go from the rather simple physical and biological unit operations to useful systems that yield biological data. The likely technological improvements will involve detection, which is currently mainly achieved by fluorescence intensity measurements.[167] In addition, fluorescent life time imaging[51,153,175] luminescence detection,[106] mass spectrometric analysis,[120–122] surface-enhanced Raman scattering detection,[168] NMR[169] and capillary electrophoresis[170] have also been demonstrated, but it would be valuable to improve detection limits and integrate a larger number of analytical methods, to expand the type of assays that can be carried out. Adding new types of assays will greatly broaden the scope of this approach.

Currently, most published work constitutes proof-of-principle experiments. Therefore the next challenge is the efficient integration of existing physical and biological unit operations. This can be more difficult than demonstrating that something works in principle (and for a limited time): issues like long-term stability and reproducibility become important, when larger numbers of experiments are analysed.

Once this is achieved the challenge may shift to defining how the results of such massive screening experiments are fed back into re-design of repeating cycles of experiments. For example, what is the best way to conduct a directed evolution experiment? At the moment we typically pick the best "needle in the haystack". This changes when quantitative information on an entire library becomes available. Such a system would, for example, allow active management between diversity and stringency in evolution cycles[171] and similar approaches could be readily applied to the other areas discussed here.

There is no reason why a human operator should make such decisions. Recently it was demonstrated that a robot scientist[172] can test hypotheses by carrying out automated laboratory experiments, evaluate the results and generate new hypotheses. The modularity of microfluidic droplet devices and high level of analytical control should eventually enable to emulate and miniaturise such bold attempts in biological automation.

The increasing diversity of published experiments in microfluidic droplets suggests that the advantages of this format are increasingly attractive to a growing circle of experimentalists and could become the method of choice for a significant fraction of biological research.

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Notes and references

1 D. S. Tawfik and A. D. Griffiths, *Nat. Biotechnol.*, 1998, **16**, 652–656.
2 A. D. Griffiths and D. S. Tawfik, *Trends Biotechnol.*, 2006, **24**, 395–402.
3 A. D. Griffiths and D. S. Tawfik, *Curr. Opin. Biotechnol.*, 2000, **11**, 338–353.
4 B. T. Kelly, J.-C. Baret, V. Taly and A. D. Griffiths, *Chem. Commun.*, 2007, 1773–1788.
5 V. Taly, B. T. Kelly and A. D. Griffiths, *ChemBioChem*, 2007, **8**, 263–272.
6 B. Rotman, *Proc. Natl. Acad. Sci. U. S. A.*, 1961, **47**, 1981–1991.
7 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.
8 F. J. Ghadessy and P. Holliger, *Protein Eng., Des. Sel.*, 2004, **17**, 201–204.
9 L. Roach, H. Song and R. Ismagilov, *Anal. Chem.*, 2005, **77**, 785–796.
10 F. Courtois, L. F. Olguin, G. Whyte, D. Bratton, W. T. S. Huck, C. Abell and F. Hollfelder, *ChemBioChem*, 2008, **9**, 439–446.
11 F. Courtois, L. F. Olguin, G. Whyte, A. B. Theberge, W. T. S. Huck, F. Hollfelder and C. Abell, *Anal. Chem.*, 2009, **81**, 3008–3016.
12 C. Holtze, A. C. Rowat, J. J. Agresti, J. B. Hutchison, F. E. Angilè, C. H. J. Schmitz, S. Köster, H. Duan, K. J. Humphry, R. A. Scanga, J. S. Johnson, D. Pisignano and D. A. Weitz, *Lab Chip*, 2008, **8**, 1632–1639.
13 J. Clausell-Tormos, D. Lieber, J.-C. Baret, A. El-Harrak, O. J. Miller, L. Frenz, J. Blouwolff, K. J. Humphry, S. Köster, H. Duan, C. Holtze, D. A. Weitz, A. D. Griffiths and C. A. Merten, *Chem. Biol.*, 2008, **15**, 427–437.
14 A. Huebner, S. Sharma, M. Srisa-Art, F. Hollfelder, J. B. Edel and A. J. deMello, *Lab Chip*, 2008, **8**, 1244–1254.
15 H. Song, D. L. Chen and R. F. Ismagilov, *Angew. Chem., Int. Ed.*, 2006, **45**, 7336–7356.
16 Y. Zhu and B. E. Power, *Adv. Biochem. Eng. Biotechnol.*, 2008, **110**, 81–114.
17 S.-Y. Teh, R. Lin, L.-H. Hung and A. P. Lee, *Lab Chip*, 2008, **8**, 198–220.
18 H. Song, J. D. Tice and R. F. Ismagilov, *Angew. Chem., Int. Ed.*, 2003, **42**, 768–772.
19 H. Song and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2003, **125**, 14613–14619.
20 A. Dove, *Nat. Biotechnol.*, 1999, **17**, 859–863.
21 D. T. Chiu, R. M. Lorenz and G. D. M. Jeffries, *Anal. Chem.*, 2009, **81**, 5111–5118.
22 D. T. Chiu and R. M. Lorenz, *Acc. Chem. Res.*, 2009, **42**, 649–658.
23 R. Williams, S. G. Peisajovich, O. J. Miller, S. Magdassi, D. S. Tawfik and A. D. Griffiths, *Nat. Methods*, 2006, **3**, 545–550.
24 M. Nakano, N. Nakai, H. Kurita, J. Komatsu, K. Takashima, S. Katsura and A. Mizuno, *J. Biosci. Bioeng.*, 2005, **99**, 293–295.
25 M. Nakano, J. Komatsu, S. Matsuura, K. Takashima, S. Katsura and A. Mizuno, *J. Biotechnol.*, 2003, **102**, 117–124.
26 A. Musyanovych, V. Mailänder and K. Landfester, *Biomacromolecules*, 2005, **6**, 1824–1828.
27 J. G. Wetmur, M. Kumar, L. Zhang, C. Palomeque, S. Wallenstein and J. Chen, *Nucleic Acids Res.*, 2005, **33**, 2615–2619.
28 F. Diehl, M. Li, Y. He, K. W. Kinzler, B. Vogelstein and D. Dressman, *Nat. Methods*, 2006, **3**, 551–559.
29 D. Dressman, H. Yan, G. Traverso, K. W. Kinzler and B. Vogelstein, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 8817–8822.
30 M. Li, F. Diehl, D. Dressman, B. Vogelstein and K. W. Kinzler, *Nat. Methods*, 2006, **3**, 95–97.
31 T. Kojima, Y. Takei, M. Ohtsuka, Y. Kawarasaki, T. Yamane and H. Nakano, *Nucleic Acids Res.*, 2005, **33**, e150.
32 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 and B. C. Godwin *et al.*, *Nature*, 2005, **437**, 376–380.
33 J. Shendure, G. J. Porreca, N. B. Reppas, X. Lin, J. P. McCutcheon, A. M. Rosenbaum, M. D. Wang, K. Zhang, R. D. Mitra and G. M. Church, *Science*, 2005, **309**, 1728–1732.
34 J. Shendure and H. Ji, *Nat. Biotechnol.*, 2008, **26**, 1135–1145.
35 E. R. Mardis, *Annu. Rev. Genomics Hum. Genet.*, 2008, **9**, 387–402.

36 A. Huebner, D. Bratton, G. Whyte, M. Yang, A. J. deMello, C. Abell and F. Hollfelder, *Lab Chip*, 2009, **9**, 692–698.
37 D. R. Link, S. L. Anna, D. A. Weitz and H. A. Stone, *Phys. Rev. Lett.*, 2004, **92**, 054503.
38 X. Niu, S. Gulati, J. B. Edel and A. J. deMello, *Lab Chip*, 2008, **8**, 1837–1841.
39 L. Fidalgo, G. Whyte, D. Bratton, C. Kaminski, C. Abell and W. T. S. Huck, *Angew. Chem., Int. Ed.*, 2008, **47**, 2042–2045.
40 http://www.raindancetechnologies.com.
41 N. Blow, *Nat. Methods*, 2009, **6**, 539–542.
42 C. Adessi, G. Matton, G. Ayala, G. Turcatti, J. J. Mermod, P. Mayer and E. Kawashima, *Nucleic Acids Res.*, 2000, **28**, 87e.
43 M. Fedurco, A. Romieu, S. Williams, I. Lawrence and G. Turcatti, *Nucleic Acids Res.*, 2006, **34**, e22.
44 P. Kumaresan, C. J. Yang, S. A. Cronier, R. G. Blazej and R. A. Mathies, *Anal. Chem.*, 2008, **80**, 3522–3529.
45 N. Beer, E. Wheeler, L. Lee-Houghton, N. Watkins, S. Nasarabadi, N. Hebert, P. Leung, D. Arnold, C. Bailey and B. Colston, *Anal. Chem.*, 2008, **80**, 1854–1858.
46 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.
47 C. A. Heid, J. Stevens, K. J. Livak and P. M. Williams, *Genome Res.*, 1996, **6**, 986–994.
48 B. Vogelstein and K. W. Kinzler, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 9236–9241.
49 P. J. Sykes, S. H. Neoh, M. J. Brisco, E. Hughes, J. Condon and A. A. Morley, *BioTechniques*, 1992, **13**, 444–449.
50 E. A. Ottesen, J. W. Hong, S. R. Quake and J. R. Leadbetter, *Science*, 2006, **314**, 1464–1467.
51 Y. Schaerli, R. C. Wootton, T. Robinson, V. Stein, C. Dunsby, M. A. A. Neil, P. M. W. French, A. J. deMello, C. Abell and F. Hollfelder, *Anal. Chem.*, 2009, **81**, 302–306.
52 K. D. Dorfman, M. Chabert, J.-H. Codarbox, G. Rousseau, P. de Cremoux and J.-L. Viovy, *Anal. Chem.*, 2005, **77**, 3700–3704.
53 M. Chabert, K. D. Dorfman, P. de Cremoux, J. Roeraade and J.-L. Viovy, *Anal. Chem.*, 2006, **78**, 7722–7728.
54 M. Kiss, L. Ortoleva-Donnelly, N. Beer, J. Warner, C. Bailey, B. Colston, J. Rothberg, D. Link and J. Leamon, *Anal. Chem.*, 2008, **80**, 8975–8981.
55 S. Mohr, Y.-H. Zhang, A. Macaskill, P. J. R. Day, R. W. Barber, N. J. Goddard, D. R. Emerson and P. R. Fielden, *Microfluid. Nanofluid.*, 2007, **3**, 611–621.
56 M. Curcio and J. Roeraade, *Anal. Chem.*, 2003, **75**, 1–7.
57 W. Wang, Z. X. Li, R. Luo, S. H. Lu, A. D. Xu and Y. J. Yang, *J. Micromech. Microeng.*, 2005, **15**, 1369–1377.
58 A. Gonzalez, D. Ciobanu, M. Sayers, N. Sirr, T. Dalton and M. Davies, *Biomed. Microdevices*, 2007, **9**, 729–736.
59 A. J. deMello, *Nature*, 2003, **422**, 28–29.
60 Y. Zhang and P. Ozdemir, *Anal. Chim. Acta*, 2009, **638**, 115–125.
61 C. Zhang and D. Xing, *Nucleic Acids Res.*, 2007, **35**, 4223–4237.
62 P. Gill and A. Ghaemi, *Nucleosides, NucleotidesNucleic Acids*, 2008, **27**, 224–243.
63 L. Mazutis, A. F. Araghi, O. J. Miller, J.-C. Baret, L. Frenz, A. Janoshazi, V. Taly, B. J. Miller, J. B. Hutchison, D. Link, A. D. Griffiths and M. Ryckelynck, *Anal. Chem.*, 2009, **81**, 4813–4821.
64 R. S. Lasken, *Curr. Opin. Microbiol.*, 2007, **10**, 510–516.
65 Y. Marcy, T. Ishoey, R. S. Lasken, T. B. Stockwell, B. P. Walenz, A. L. Halpern, K. Y. Beeson, S. M. D. Goldberg and S. R. Quake, *PLoS Genet.*, 2007, **3**, 1702–1708.
66 H. Leemhuis, V. Stein, A. D. Griffiths and F. Hollfelder, *Curr. Opin. Struct. Biol.*, 2005, **15**, 472–478.
67 M. J. Olsen, J. Gam, B. L. Iverson and G. Georgiou, *Methods Mol. Biol.*, 2003, **230**, 329–342.
68 F. J. Ghadessy, J. L. Ong and P. Holliger, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 4552–4557.
69 M. d'Abbadie, M. Hofreiter, A. Vaisman, D. Loakes, D. Gasparutto, J. Cadet, R. Woodgate, S. Pääbo and P. Holliger, *Nat. Biotechnol.*, 2007, **25**, 939–943.
70 F. J. Ghadessy and P. Holliger, *Methods Mol. Biol. (Totowa, N. J.)*, 2007, **352**, 237–248.
71 F. J. Ghadessy, N. Ramsay, F. Boudsocq, D. Loakes, A. Brown, S. Iwai, A. Vaisman, R. Woodgate and P. Holliger, *Nat. Biotechnol.*, 2004, **22**, 755–759.
72 J. L. Ong, D. Loakes, S. Jaroslawski, K. Too and P. Holliger, *J. Mol. Biol.*, 2006, **361**, 537–550.
73 K. Bernath, M. Hai, E. Mastrobattista, A. D. Griffiths, S. Magdassi and D. S. Tawfik, *Anal. Biochem.*, 2004, **325**, 151–157.
74 E. Mastrobattista, V. Taly, E. Chanudet, P. Treacy, B. T. Kelly and A. D. Griffiths, *Chem. Biol.*, 2005, **12**, 1291–1300.
75 A. Aharoni, G. Amitai, K. Bernath, S. Magdassi and D. S. Tawfik, *Chem. Biol.*, 2005, **12**, 1281–1289.
76 A. D. Griffiths and D. S. Tawfik, *EMBO J.*, 2003, **22**, 24–35.
77 A. Sepp, D. S. Tawfik and A. D. Griffiths, *FEBS Lett.*, 2002, **532**, 455–458.
78 O. Nord, M. Uhlén and P.-A. Nygren, *J. Biotechnol.*, 2003, **106**, 1–13.
79 R. Gan, Y. Yamanaka, T. Kojima and H. Nakano, *Biotechnol. Prog.*, 2008, **24**, 1107–1114.
80 N. Doi and H. Yanagawa, *FEBS Lett.*, 1999, **457**, 227–230.
81 J. Bertschinger and D. Neri, *Protein Eng., Des. Sel.*, 2004, **17**, 699–707.
82 V. Stein, I. Sielaff, K. Johnsson and F. Hollfelder, *ChemBioChem*, 2007, **8**, 2191–2194.
83 G. L. Verdine and L. D. Walensky, *Clin. Cancer Res.*, 2007, **13**, 7264–7270.
84 L. D. Walensky, A. L. Kung, I. Escher, T. J. Malia, S. Barbuto, R. D. Wright, G. Wagner, G. L. Verdine and S. J. Korsmeyer, *Science*, 2004, **305**, 1466–1470.
85 D. Lipovsek and A. Plückthun, *J. Immunol. Methods*, 2004, **290**, 51–67.
86 S. A. Gai and K. D. Wittrup, *Curr. Opin. Struct. Biol.*, 2007, **17**, 467–473.
87 G. Smith and V. Petrenko, *Chem. Rev.*, 1997, **97**, 391–410.
88 J. Q. Boedicker, L. Li, T. R. Kline and R. F. Ismagilov, *Lab Chip*, 2008, **8**, 1265–1272.
89 A. Grodrian, J. Metze, T. Henkel, K. Martin, M. Roth and J. M. Köhler, *Biosens. Bioelectron.*, 2004, **19**, 1421–1428.
90 A. Huebner, L. F. Olguin, D. Bratton, G. Whyte, W. T. S. Huck, A. J. deMello, J. B. Edel, C. Abell and F. Hollfelder, *Anal. Chem.*, 2008, **80**, 3890–3896.
91 A. Huebner, M. Srisa-Art, D. Holt, C. Abell, F. Hollfelder, A. J. deMello and J. B. Edel, *Chem. Commun.*, 2007, 1218–1220.
92 K. Martin, T. Henkel, V. Baier, A. Grodrian, T. Schön, M. Roth, J. M. Köhler and J. Metze, *Lab Chip*, 2003, **3**, 202–207.
93 C. Luo, X. Yang, Q. Fu, M. Sun, Q. Ouyang, Y. Chen and H. Ji, *Electrophoresis*, 2006, **27**, 1977–1983.
94 C. H. J. Schmitz, A. C. Rowat, S. Köster and D. A. Weitz, *Lab Chip*, 2009, **9**, 44–49.
95 M. Chabert and J.-L. Viovy, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3191–3196.
96 J. F. Edd, D. D. Carlo, K. J. Humphry, S. Köster, D. Irimia, D. A. Weitz and M. Toner, *Lab Chip*, 2008, **8**, 1262–1264.
97 M. He, J. S. Edgar, G. D. M. Jeffries, R. M. Lorenz, J. P. Shelby and D. T. Chiu, *Anal. Chem.*, 2005, **77**, 1539–1544.
98 S. Köster, F. E. Angilè, H. Duan, J. J. Agresti, A. Wintner, C. Schmitz, A. C. Rowat, C. A. Merten, D. Pisignano, A. D. Griffiths and D. A. Weitz, *Lab Chip*, 2008, **8**, 1110–1115.
99 A. E. Sgro, P. B. Allen and D. T. Chiu, *Anal. Chem.*, 2007, **79**, 4845–4851.
100 C. Kim, K. S. Lee, Y. E. Kim, K.-J. Lee, S. H. Lee, T. S. Kim and J. Y. Kang, *Lab Chip*, 2009, **9**, 1294–1297.
101 Y. Zhan, J. Wang, N. Bao and C. Lu, *Anal. Chem.*, 2009, **81**, 2027–2031.
102 J.-C. Baret, O. J. Miller, V. Taly, M. Ryckelynck, A. El-Harrak, L. Frenz, C. Rick, M. L. Samuels, J. B. Hutchison, J. J. Agresti, D. R. Link, D. A. Weitz and A. D. Griffiths, *Lab Chip*, 2009, **9**, 1850–1858.
103 P. S. Dittrich, M. Jahnz and P. Schwille, *ChemBioChem*, 2005, **6**, 811–814.
104 N. Damean, L. F. Olguin, F. Hollfelder, C. Abell and W. T. S. Huck, *Lab Chip*, 2009, **9**, 1707–1713.
105 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.
106 A. Liau, R. Karnik, A. Majumdar and J. H. D. Cate, *Anal. Chem.*, 2005, **77**, 7618–7625.

107 L. Frenz, K. Blank, E. Brouzes and A. D. Griffiths, *Lab Chip*, 2009, **9**, 1344–1348.
108 H. N. Joensson, M. L. Samuels, E. R. Brouzes, M. Medkova, M. Uhlén, D. R. Link and H. Andersson-Svahn, *Angew. Chem., Int. Ed.*, 2009, **48**, 2518–2521.
109 Z. Han, W. Li, Y. Huang and B. Zheng, *Anal. Chem.*, 2009, **81**, 5840–5845.
110 E. Brouzes, M. Medkova, N. Savenelli, D. Marran, M. Twardowski, J. B. Hutchison, J. M. Rothberg, D. R. Link, N. Perrimon and M. L. Samuels, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 14195–14200.
111 H. Z. Chen and G. Zubay, *Methods Enzymol.*, 1983, **101**, 674–690.
112 T. Nisisako and T. Torii, *Lab Chip*, 2008, **8**, 287–293.
113 S. Bershtein and D. S. Tawfik, *Curr. Opin. Chem. Biol.*, 2008, **12**, 151–158.
114 S. Bershtein, K. Goldin and D. S. Tawfik, *J. Mol. Biol.*, 2008, **379**, 1029–1044.
115 S. Bershtein, M. Segal, R. Bekerman, N. Tokuriki and D. S. Tawfik, *Nature*, 2006, **444**, 929–932.
116 H. L. Steele, K.-E. Jaeger, R. Daniel and W. R. Streit, *J. Mol. Microbiol. Biotechnol.*, 2009, **16**, 25–37.
117 M. Ferrer, F. Martínez-Abarca and P. N. Golyshin, *Curr. Opin. Biotechnol.*, 2005, **16**, 588–593.
118 W. R. Streit and R. A. Schmitz, *Curr. Opin. Microbiol.*, 2004, **7**, 492–498.
119 T. K. Garyantes, *Drug Discovery Today*, 2002, **7**, 489–490.
120 L. M. Fidalgo, G. Whyte, B. T. Ruotolo, J. L. P. Benesch, F. Stengel, C. Abell, C. V. Robinson and W. T. S. Huck, *Angew. Chem., Int. Ed.*, 2009, **48**, 3665–3668.
121 J. Pei, Q. Li, M. S. Lee, G. A. Valaskovic and R. T. Kennedy, *Anal. Chem.*, 2009, **81**, 6558–6561.
122 T. Hatakeyama, D. L. Chen and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2006, **128**, 2518–2519.
123 S. Melkko, C. E. Dumelin, J. Scheuermann and D. Neri, *Drug Discovery Today*, 2007, **12**, 465–471.
124 M. Han, X. Gao, J. Z. Su and S. Nie, *Nat. Biotechnol.*, 2001, **19**, 631–635.
125 B. J. Battersby, G. A. Lawrie, A. P. R. Johnston and M. Trau, *Chem. Commun.*, 2002, 1435–1441.
126 J. S. Edgar, G. Milne, Y. Zhao, C. P. Pabbati, D. S. W. Lim and D. T. Chiu, *Angew. Chem., Int. Ed.*, 2009, **48**, 2719–2722.
127 B. Zheng, L. S. Roach and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2003, **125**, 11170–11171.
128 B. Zheng, J. D. Tice and R. F. Ismagilov, *Anal. Chem.*, 2004, **76**, 4977–4982.
129 P. S. Dittrich and A. Manz, *Nat. Rev. Drug Discovery*, 2006, **5**, 210–218.
130 B. Zheng, C. J. Gerdts and R. F. Ismagilov, *Curr. Opin. Struct. Biol.*, 2005, **15**, 548–555.
131 J. Leng and J.-B. Salmon, *Lab Chip*, 2009, **9**, 24–34.
132 J. Shim, L. F. Olguin, G. Whyte, D. Scott, A. Babtie, C. Abell, W. T. S. Huck and F. Hollfelder, *J. Am. Chem. Soc.*, 2009, DOI: 10.1021/ja904823.
133 H. Hufnagel, A. Huebner, C. Gülch, K. Güse, C. Abell and F. Hollfelder, *Lab Chip*, 2009, **9**, 1576–1582.
134 B. S. Edwards, S. M. Young, I. Ivnitsky-Steele, R. D. Ye, E. R. Prossnitz and L. A. Sklar, *Methods Mol. Biol.*, 2009, **486**, 151–165.
135 M. Fernández-Suárez and A. Y. Ting, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 929–943.
136 K. Johnsson, *Nat. Chem. Biol.*, 2009, **5**, 63–65.
137 A. E. Carpenter, *Methods Mol. Biol.*, 2009, **486**, 193–211.
138 U. S. Eggert and T. J. Mitchison, *Curr. Opin. Chem. Biol.*, 2006, **10**, 232–237.
139 M. E. Lidstrom and D. R. Meldrum, *Nat. Rev. Microbiol.*, 2003, **1**, 158–164.
140 L. Cai, N. Friedman and X. S. Xie, *Nature*, 2006, **440**, 358–362.
141 P. J. Choi, L. Cai, K. Frieda and X. S. Xie, *Science*, 2008, **322**, 442–446.
142 X. S. Xie, P. J. Choi, G.-W. Li, N. K. Lee and G. Lia, *Annu. Rev. Biophys.*, 2008, **37**, 417–444.
143 J. C. Gadd, C. L. Kuyper, B. S. Fujimoto, R. W. Allen and D. T. Chiu, *Anal. Chem.*, 2008, **80**, 3450–3457.
144 D. Chen, W. Du, Y. Liu, W. Liu, A. Kuznetsov, F. E. Mendez, L. H. Philipson and R. F. Ismagilov, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 16843–16848.
145 J. M. Köhler and T. Henkel, *Appl. Microbiol. Biotechnol.*, 2005, **69**, 113–125.
146 N. Goshima, Y. Kawamura, A. Fukumoto, A. Miura, R. Honma, R. Satoh, A. Wakamatsu, J. Yamamoto, K. Kimura, T. Nishikawa, T. Andoh, Y. Iida, K. Ishikawa, E. Ito and N. Kagawa *et al.*, *Nat. Methods*, 2008, **5**, 1011–1017.
147 J. R. Porter, C. I. Stains, B. W. Jester and I. Ghosh, *J. Am. Chem. Soc.*, 2008, **130**, 6488–6497.
148 S. W. Michnick, *Curr. Opin. Biotechnol.*, 2003, **14**, 610–617.
149 N. Johnsson and K. Johnsson, *ChemBioChem*, 2003, **4**, 803–810.
150 M. Srisa-Art, A. J. deMello and J. B. Edel, *Anal. Chem.*, 2007, **79**, 6682–6689.
151 M. Srisa-Art, E. C. Dyson, A. J. deMello and J. B. Edel, *Anal. Chem.*, 2008, **80**, 7063–7067.
152 M. Srisa-Art, D.-K. Kang, J. Hong, H. Park, R. J. Leatherbarrow, J. B. Edel, S.-I. Chang and A. J. deMello, *ChemBioChem*, 2009, **10**, 1605–1611.
153 M. Srisa-Art, A. J. deMello and J. B. Edel, *Phys. Rev. Lett.*, 2008, **101**, 014502.
154 Y. Zhang, V. Bailey, C. M. Puleo, H. Easwaran, E. Griffiths, J. G. Herman, S. B. Baylin and T.-H. Wang, *Lab Chip*, 2009, **9**, 1059–1064.
155 H. Song, H.-W. Li, M. Munson, T. VanHa and R. Ismagilov, *Anal. Chem.*, 2006, **78**, 4839–4849.
156 S. A. Benner and A. M. Sismour, *Nat. Rev. Genet.*, 2005, **6**, 533–543.
157 P. M. Boyle and P. A. Silver, *J. R. Soc. Interface*, 2009, **6**(Suppl 4), S535–S546.
158 S. Gulati, V. Rouilly, X. Niu, J. Chappell, R. I. Kitney, J. B. Edel, P. S. Freemont and A. J. deMello, *J. R. Soc. Interface*, 2009, **6**(Suppl 4), S493–S506.
159 A. M. Sismour, S. Lutz, J.-H. Park, M. J. Lutz, P. L. Boyer, S. H. Hughes and S. A. Benner, *Nucleic Acids Res.*, 2004, **32**, 728–735.
160 K. Wang, H. Neumann, S. Y. Peak-Chew and J. W. Chin, *Nat. Biotechnol.*, 2007, **25**, 770–777.
161 O. Rackham and J. W. Chin, *Nat. Chem. Biol.*, 2005, **1**, 159–166.
162 J. Xie and P. G. Schultz, *Curr. Opin. Chem. Biol.*, 2005, **9**, 548–554.
163 M. Ohuchi, H. Murakami and H. Suga, *Curr. Opin. Chem. Biol.*, 2007, **11**, 537–542.
164 K. Brenner, L. You and F. H. Arnold, *Trends Biotechnol.*, 2008, **26**, 483–489.
165 S. Hooshangi and W. E. Bentley, *Curr. Opin. Biotechnol.*, 2008, **19**, 550–555.
166 D. N. Breslauer, P. J. Lee and L. P. Lee, *Mol. BioSyst.*, 2006, **2**, 97–112.
167 J.-L. Reymond, V. S. Fluxà and N. Maillard, *Chem. Commun.*, 2009, 34–46.
168 G. Wang, C. Lim, L. Chen, H. Chon, J. Choo, J. Hong and A. J. deMello, *Anal. Bioanal. Chem.*, 2009, **394**, 1827–1832.
169 R. A. Kautz, W. K. Goetzinger and B. L. Karger, *J. Comb. Chem.*, 2005, **7**, 14–20.
170 J. S. Edgar, C. P. Pabbati, R. M. Lorenz, M. He, G. S. Fiorini and D. T. Chiu, *Anal. Chem.*, 2006, **78**, 6948–6954.
171 S. Bershtein and D. S. Tawfik, *Mol. Biol. Evol.*, 2008, **25**, 2311–2318.
172 R. D. King, J. Rowland, S. G. Oliver, M. Young, W. Aubrey, E. Byrne, M. Liakata, M. Markham, P. Pir, L. N. Soldatova, A. Sparkes, K. E. Whelan and A. Clare, *Science*, 2009, **324**, 85–89.
173 R. B. Fair, *Microfluid. Nanofluid.*, 2007, **3**, 245–281.
174 M. Abdelgawad and A. R. Wheeler, *Adv. Mater.*, 2009, **21**, 920–925.
175 T. Robinson, Y. Schaerli, R. C. Wootton, F. Hollfelder, C. Dunsby, G. S. Baldwin, M. A. A. Neil, P. M. W. French and A. J. deMello, *Lab Chip*, 2009, DOI: 10.1039/b913293g.

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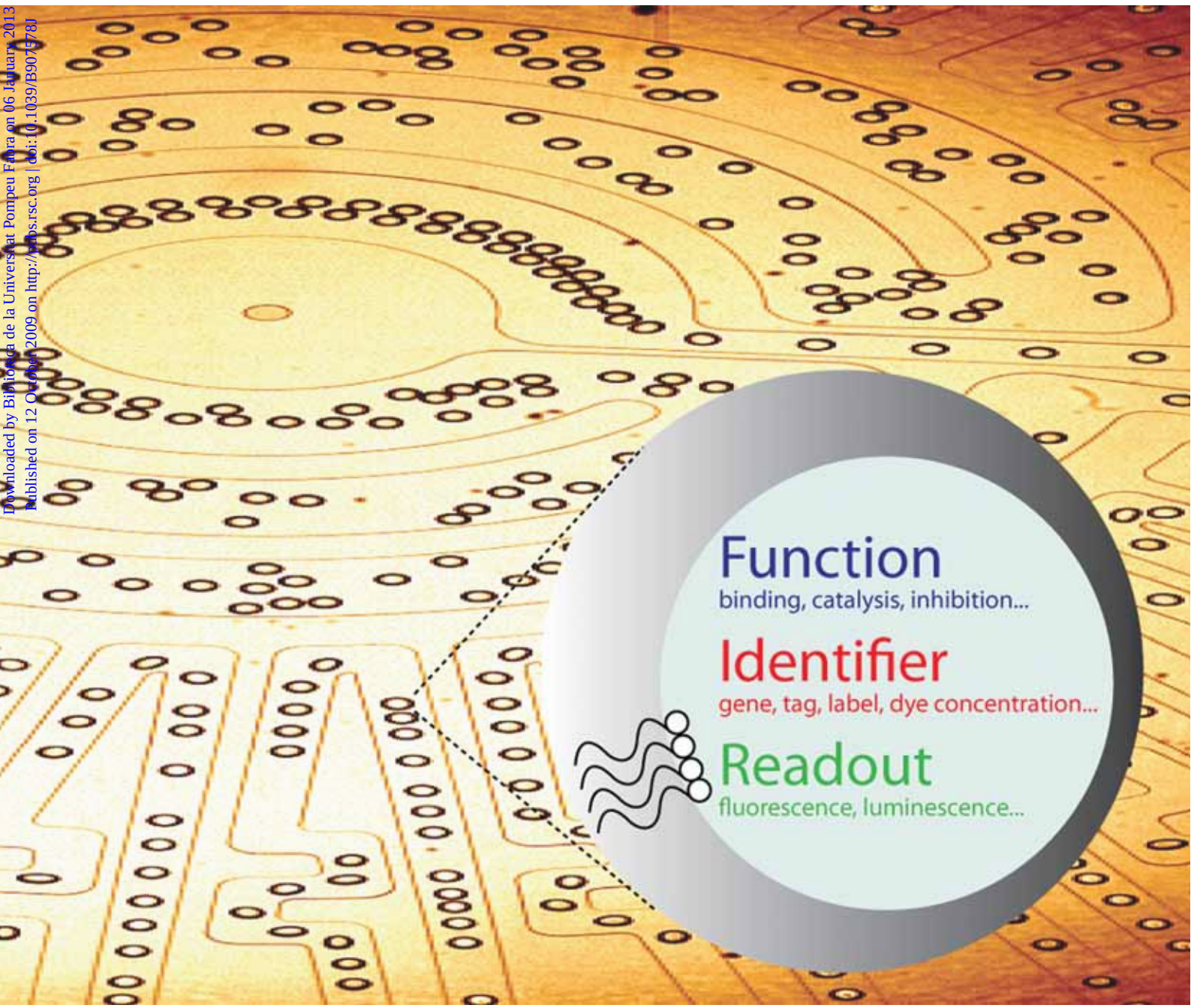

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HIGHLIGHT

Florian Hollfelder *et al.*

The potential of microfluidic water-in-oil droplets in experimental biology