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Invited Mini Review

Recent advances in microfluidic technologies for biochemistry and molecular biology

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Advances in the fields of proteomics and genomics have necessitated the development of high-throughput screening methods (HTS) for the systematic transformation of large amounts of biological/chemical data into an organized database of knowledge. Microfluidic systems are ideally suited for high-throughput biochemical experimentation since they offer high analytical throughput, consume minute quantities of expensive biological reagents, exhibit superior sensitivity and functionality compared to traditional micro-array techniques and can be integrated within complex experimental work flows. A range of basic biochemical and molecular biological operations have been transferred to chip-based microfluidic formats over the last decade, including gene sequencing, emulsion PCR, immunoassays, electrophoresis, cell-based assays, expression cloning and macromolecule blotting. In this review, we highlight some of the recent advances in the application of microfluidics to biochemistry and molecular biology. [BMB reports 2011; 44(11): 705-712]

INTRODUCTION: BIOCHEMISTRY AND MOLECULAR BIOLOGY FOR MICROFLUIDICS

Over the past 60 years, the fields of biochemistry and molecular biology have enjoyed tremendous developments in terms of both the basic science and associated enabling technologies. The ever increasing biochemical information that has accompanied the advent of the postgenomic era has led to a need for high throughput experimentation and sensitive analytical systems. Conventional experimental tools suffer from large reagent consumption, insufficient throughput, and errors related to the transfer of sample between multiple instruments (1, 2).

In recent years, microfluidic technologies have emerged as potential high throughput platforms for performing various biochemical and molecular biological experiments. Microfluidic systems are able to process small volumes of fluid using channels with dimensions ranging from hundreds of nanometers to hundreds of micrometres (3). Microfluidic systems offer numerous advantages over conventional analytical devices, including faster analysis times, superior analytical performance, reduced sample/reagent consumption, reduction of instrument footprints, portability, and facile monolithic integration of functional components (2, 4, 5).

Genes and proteins are the fundamental building blocks of life and have been at the heart of developments in molecular biology. In this review, we briefly highlight selected developments in microfluidic technologies for biochemistry and molecular biology, with a particular emphasis on genomic and proteomic research.

MICROFLUIDIC TOOLS FOR GENOMIC ANALYSIS

Rapid developments of microfluidic technologies for DNA sequencing, synthesis, amplification, modification and translation have occurred over the last decade. Particular emphasis has been placed on the realisation of high throughput sequencing, single copy and digital PCR and the integration of functional components for novel applications in personalised healthcare and biomolecule production.

DNA sequencing technologies

DNA sequencing is the central activity in genomic science. The rapid development of sequencing technology was instrumental to the early completion of the Human Genome Project, and will undoubtedly continue to play an important role in molecular biology and biochemistry for many years to come. Various microfluidic tools for DNA sequencing have been successfully transferred to planar-chip formats (6-9). For example, Quake and co-workers sequenced DNA with a 4 base pair read length using a sequencing-by-synthesis paradigm (6). The microfluidic module can be easily integrated with other functional components and in theory read lengths can be significantly extended through improvements to both the surface chemistry and polymerase activity.

Sanger sequencing, due to its long read lengths and flexibility

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in scale, continues to represent the best option for de novo sequencing of complex new genomes. A range of microfluidic approaches for Sanger sequencing have been reported in the literature (7-11) with the most sophisticated and established technologies being presented by Richard Mathies at the University of California, Berkeley (8, 9). For example, Blazej *et al*. reported a nanoliter-scale microfabricated bioprocessor integrating all three Sanger sequencing steps (9). In this study, the authors demonstrated complete Sanger sequencing from only 1 fmol of DNA template. Additionally, a sensitivity of 100 attomole was achieved by the same group by incorporating a gel based affinity method for DNA capture, concentration, and inline injection with the Sanger sequencing microfluidic platform (8).

Although DNA sequencing platforms for microfluidic devices are still non-optimised in terms of throughput and performance, it is expected that they will play an important role in low cost, medium throughput personal sequencing applications in the future (2). Moreover, such tools will be invaluable in enabling single cell genomics for studying the genetic heterogeneity of somatic cells, that are currently unattainable using conventional methods (12).

DNA synthesis

A few proof-of-concept microfluidic oligonucleotide synthesizers have been developed. For example, Kong and co-workers synthesized 1 kb long genes from minute (10-25 nM) concentrations of oligonucleotides within a multi-chamber device, using two orders of magnitude less reagent than in conventional approaches (13). More recently, Lee *et al*. demonstrated the synthesis of 16 oligonucleotides on a programmable microfluidic synthesis platform and used these sequences to assemble a DNA construct of ~200 base pairs long (14). The microfluidic architecture incorporates integrated valves and thus allows individual manipulation and collection of products. Compared to conventional synthesizers, the cost of gene synthesis is reduced by two orders of magnitude whilst maintaining the synthesis scale. Finally, Church and co-workers reported the use of an integrated chip-based system, that combines selective oligonucleotide pool amplification, optimized gene assembly and enzymatic error correction for highly parallel gene synthesis (15). The authors were able to assemble 47 genes encoding a total of ~35 kbp of DNA from a complex background containing 13,000 oligonucleotides. Further improvements in this regard are expected as more compatible materials and surface coatings are made available.

DNA amplification by polymerase chain reaction

DNA amplification via the polymerase chain reaction (PCR) is an essential tool in forensics, diagnostics, cloning, and sequencing (17). The adaptation of microfluidic formats for thermal cycling has proved to be significant in terms of reducing reagent consumption, lowering amplification times through efficient thermal transport, increasing analytical throughput and allowing integration with post-reaction sample analysis. Of particular note was the demonstration of continuous flow PCR within an extended microfluidic channel (18). In this study, a 20 cycle amplification of a 176 bp fragment was performed in times as low as 90 seconds. More recently, significant developments in chip-based PCR have been afforded through the adoption of segmented-flow or droplet-based formats. Such systems, in principle, allow millions of individual amplification reactions to be performed within a few minutes and more importantly enable single-copy and digital PCR. Use of segmented flows excludes the deleterious interactions of channel walls with polymerase and template DNA, improving reaction yield and preventing cross contamination of samples. For example, Florian Hollfelder and co-workers reported continuous flow PCR within pL-volume droplets (16). The high efficiency of the approach was shown to allow for amplification at the single molecule of DNA per droplet level. Specifically, an off-chip heating system was used to generate a radial thermal gradient, and thermal cycling was achieved by passing droplets through spatially separated zones for denaturation, annealing and template extension as shown in Fig. 1. Using this system, an 85 base-pair long template was successfully amplified with amplification factors in excess of 5×10^6. Using a similar approach, Tewhey *et al*. achieved targeted enrichment of specific human genome loci in microdroplets performing 1.5 million amplifications in parallel (19).

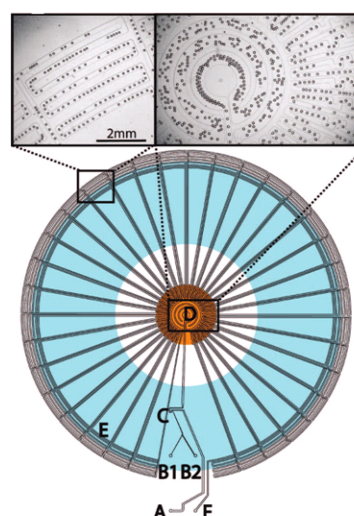

Fig. 1. The design of the radial emulsion PCR device where microfluidic droplets move through a temperature gradient. The device contains an oil inlet (A) that joins two aqueous inlet channels (B1 and B2) to generate droplets (C). The droplets pass through the inner circles in the hot zone (D) for initial denaturation of the template and travel on to the periphery for primer annealing and template extension (E). The droplets then flow back to the centre, where the DNA is denatured and a new cycle begins. 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, respectively (16). Reproduced with permission from (16). Copyright 2009 American Chemical Society.

Directed evolution for genetic modification

In vitro directed evolution is generic method used to evolve proteins or RNA with specific properties not found in nature. In simple terms, a gene encoding a protein of interest is randomly mutated to generate a library of gene variants using DNA shuffling or error-prone PCR. The library is then screened for the presence of mutants possessing a desired characteristic. Variants identified in the screen are then amplified and sequenced, establishing what sequence mutations have occurred. Based on the information gathered, further rounds of directed evolution are performed where the selected mutants of the previous round are diversified to create a new library. At the end of multiple rounds, all evolved protein or RNA mutants can be characterized using biochemical methods. The use of droplet-based microfluidic systems for performing directed evolution is particularly advantageous for many reasons. First each reaction can be encapsulated and performed in a pL-volume reaction vessel. Second high-throughput sorting of droplets (based on their phenotype) is possible, and finally compartmentalization of cells limits the diffusion of molecules away from the cell and allows efficient detection of enzymatic activity. Recently, Agresti *et al*. demonstrated directed evolution in a droplet based microfluidic system that incorporated droplet generation, incubation, and fluorescence activated droplet sorting (20). After 2 rounds of mutagenesis and screening, the authors were able to identify several significantly improved mutants with some approaching theoretical diffusion limited efficiencies. In total, approximately 108 individual enzyme reactions were screened in 10 hours using less than 150 μl of reagent. This represents a 1000-fold increase in speed and a 1-million-fold reduction in cost compared to state-of-the-art robotic screening systems.

MICROFLUIDIC TOOLS FOR PROTEOMIC ANALYSIS

Due to the enormous complexity exhibited by common proteomic samples, analytical platforms capable of extracting and processing large amounts of information in a sensitive and high throughput manner are required (21). The ability to study protein structure, function and interactions is further complicated by the fact that thousands of individual proteins may be present at widely varying abundance levels. In recent years, a range of microfluidic tools for performing efficient protein analysis have been reported. For example, protein crystallization (22-25), protein-protein interactions (26-28), and two-dimensional gel electrophoresis (29, 30) have been realised within planar-chip formats.

Protein structure

Protein crystallization is the basic technique required to elucidate the three-dimensional structure of a diversity of molecules and complexes (31). The screening of crystallisation conditions is an essential but laborious task. This involves finding buffer conditions that allow the formation of protein crystal populations of low polydispersity. On the macroscale, this screening process is often prohibitively long and requires the use of expensive robotic systems. Since protein samples are expensive and rare, it is advantageous to screen protein crystallization conditions using minimal amounts of reagents. Protein crystallization screening in small-volume droplet is particularly advantageous in this respect since only tiny reagent volumes are required, interaction of precipitates with the channel walls is prevented along with cross contamination between experiments. Rustem Ismagilov and co-workers have pioneered protein crystallization screening in droplet-based microfluidic systems (22-25, 32). In basic terms, nanoliter-volume droplets containing different compositions of protein and precipitants can be rapidly generated, stored and incubated allowing crystal formation to be monitored (22). The same group then refined the basic method to incorporate controlled vapor diffusion. Composite microfluidic devices were shown to be successful in performing protein crystallization trials in nanoliter aqueous droplets where the crystal quality could be directly assessed by on-chip X-ray diffraction (23). More recently, the same group were able to obtain enough crystals to solve de novo the X-ray crystal structure of oligoendopeptidase F by separating the nucleation and the growth step within a droplet-based microfluidic reactor (24). Additionally, the same approach has been used to crystallize membrane proteins (25).

Protein functionality

A variety of enzymatic assays, protein interaction studies, and immunoassays have been performed in microfluidic devices to determine protein functions, identity and quantity. Exemplar studies are now discussed.

Enzymatic assays: Enzymes play a key role in many cellular processes such as signaling pathways, metabolism, and gene expression. In particular, cell based enzymatic activity assays have been widely used in microfluidic devices, because it is possible to minimize or prevent diffusion of molecules away from the cells (allowing studies down to the single cell level), reduce costs through reduction of reagent usage, improve throughput, allow novel perfusion patterns, and improve temporal resolution (33-35). For example, microfluidic systems have been used to study immune response from macrophages (36), metabolic secretions from preimplantation of embryos (37), glycerol secretion from cultured adipocytes (38), and activity of expressed enzymes from prokaryotic cells (39, 40). In continuous flow formats, cell culturing systems are often coupled with enzymatic activity detection to provide real-time monitoring of secreted molecules such as nitric oxide (36) and glycerol (38). Such an approach can provide useful information regarding cellular responses to an external stimulus and gives valuable insights into cellular metabolism. Droplet-based microfluidic systems have also been utilized for the detection of expressed enzymes from *Escherichia coli* cells. Droplet-based systems are especially advantageous for the detection of secreted molecules, because it is possible to assess enzyme turnover by preventing the diffusion of secreted molecules away from the cell. Indeed, the compartmentalization of single cells in pL-nL droplets enables the study of cell heterogeneity in a high throughput manner (39-41). In an elegant

study by Hollfelder and co-workers, the expression of the enzyme alkaline phosphatase in droplets allowed extraction of enzymatic activity kinetics in *E. coli* cells (39). Additionally, the same group have since investigated the time dependence of protein expression of monomeric red fluorescent protein and the enzymatic activity of co-expressed alkaline phosphatase in compartmentalized *E. coli* cells (40).

Protein-protein interaction: Protein-protein interactions are critical for many biological functions such as signal mediation between the exterior and interior of cells. Development of high-throughput screening is crucial for probing protein-protein interactions due to the large number of proteins present in biological systems. For example, even a small bacterial genome contains a few thousand proteins with millions of potential protein-protein interactions (28). Using microfluidics, it is possible to screen large numbers of proteins with minimal consumption in a high-throughput manner (26-28). For example, Beebe and coworkers demonstrated the use of hydrogels to probe protein-protein interactions using fluorescence resonance energy transfer (27). More recently, Srisa-Art *et al*. reported the interaction of angiogenin (ANG) with anti-ANG antibodies using FRET in microdroplets to extract high-precision binding kinetics (26). Fig. 2 shows the schematic diagram of FRET detection in microdroplets. Finally, Stephen Quake and collegues performed exhaustive measurements on the protein-protein interactions of 43 *Streptococcus pneumonia* proteins (28). Their protein interaction network generator combines on-chip protein synthesis with an in situ microfluidic affinity array to generate a protein interaction network. The authors found that the resulting network of 157 interactions was denser than that of already known networks which suggested hypotheses concerning feedback within various metabolic pathways.

Immunoassays: Immunoassays exploit the sensitivity and specificity of antibody-antigen interactions for the detection of relevant analytes (42, 43). Immunoassays are used for biological and chemical analysis with applications in infectious disease diagnostics, immunology, detection of biological warfare agents, and environmental diagnostics (42). Conventional immunoassays suffer from slow response times and sensitivity limitations due to diffusion of the antigen to the immobilized antibody. This leads to long incubation times and thus limits analytical-throughput. Microfluidic systems for performing immunoassays offer several advantages such as high surface area to volume ratios, reduced consumption of reagents and improved throughput and reproducibility through automation.

Immunoassays can be divided into 2 major types: heterogeneous and homogeneous. Heterogeneous immunoassays employ solid supports, while antibodies and antigens interact in free solution in homogeneous immunoassays. Both types of immunoassays have been transferred to microfluidic systems. Delamarche *et al*. first introduced the use of microfluidic channel networks for surface patterning of immunoglobulin in high throughput, parallel immunoassays (44). It should also be noted that microbeads have often been used in microfluidic systems

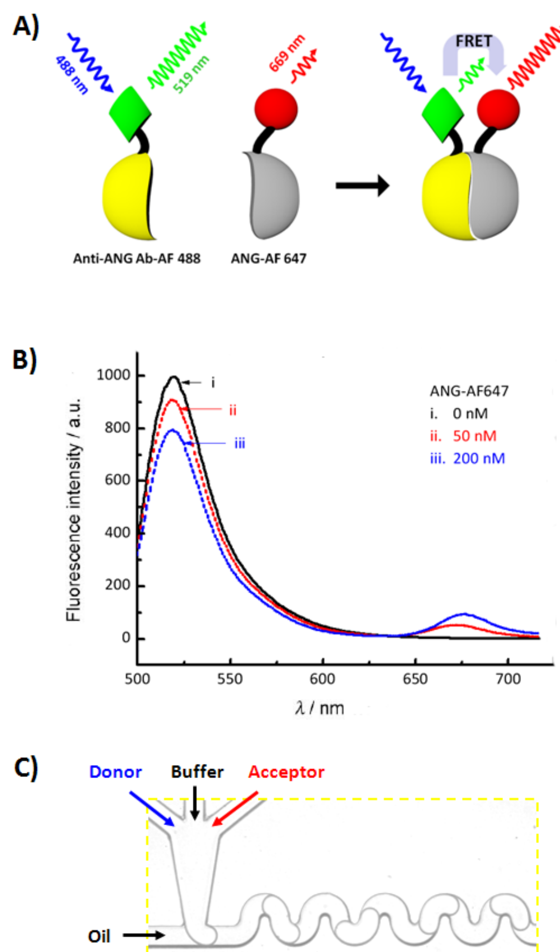

Fig. 2. (A) Schematic of FRET facilitated by protein-protein binding. (B) Fluorescence emission spectra of a mixture of anti-ANG Ab-AF488 and ANG AF647. The anti-ANG Ab-AF488 concentration was fixed at 10 nM while the ANG-AF647 concentration was varied (0, 50 and 200 nM). (C) Image of droplets generated within the microfluidic device (26). Reproduced with permission from (26). Copyright 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

for heterogeneous immunoassays (36, 45-48). More recently, Yang *et al*. demonstrated the use of superporous agarose beads as solid supports for detecting goat IgG (49). In this study, the porosity of the beads is important since it lowers the fluidic resistance and increases the active surface area, enhancing assay sensitivity. Homogeneous immunoassays have been demonstrated in a number of studies. For example, Harrison and co-workers pioneered the development of microfluidic devices based on capillary electrophoresis (50, 51). This approach allowed integration of 6 functional electrophoresis manifolds on a single chip and achieved simultaneous quantification of anti-estradiol and ovalbumin in less than 1 minute (50). Recently, gel-electrophoresis immunoassay microchip with integrated

sample pretreatment and electrophoresis was demonstrated by Herr *et al*. (52). Using this system, the authors demonstrate rapid (<10 minutes) measurement of the collagen cleaving enzyme matrix metalloproteinase-8 using just 20 μl of saliva.

Protein profiling

It is necessary to spatially separate analytes efficiently for high throughput analysis of protein expression. In this regard, chip-based separation formats are especially powerful (54, 55). Advantages of performing electrophoresis on the microscale include improved analytical performance (with respect to both efficiency and resolution), lower sample consumption, system portability, and ultra-short analysis times. In addition, it is simple to integrate capillary electrophoresis with both up-stream and down-stream components such as post column reactors and mass spectroscopic analysis (30). For example, a monolithically integrated capillary electrophoresis-electrospraying ionization microchip was reported by Sikanen *et al*. to allow mass spectrometric analysis of small molecules and peptides (30). Free flow electrophoresis is a continuous separation technique that utilizes hydrodynamic flow and an applied electric field perpendicular to the flow to separate analytes in flowing streams (54). Recent developments of this method include studies by Bowser and co-workers on the use of free flow electrophoresis in the separation of mitochondria and primary amines (56-58). Zalewski *et al*. demonstrated synchronized, continuous flow zone electrophoresis on a microfluidic device for the first time (59). In this study, the hydrodynamic flow was driven electrokinetically in addition to the orthogonal separation field. As proof of concept, the authors separated Rhodamine B and fluorescein as well as a 3 component mixture of fluorescein, rhodamine B, and rhodamine 6 G.

Finally, a number of microfluidic tools for performing two-dimensional separations have been reported in recent years. Recently, Herr and colleagues demonstrated automated 2D electrophoresis for protein immunoblotting applications using photo-patterned polyacrylamide gels in glass microfluidic devices (29, 53, 60). The authors demonstrated rapid on-chip polyacrylamide gel electrophoresis (PAGE) for the first time, and a model sample of fluorescently labelled BSA (α-actinin) and prostate specific antigen was selected to develop and characterize the assay (29). In another study, the authors integrated PAGE, transfer, and antibody-functionalized blotting regions in one continuous assay to directly map protein mobility to antibody binding. To demonstrate the efficacy of the approach, a complete native immunoblot of free prostate specific antigen from human seminal fluid was performed in less than 5 minutes as shown in Fig. 3 (53). Using a rather different approach for coupling orthogonal separations, Niu *et al*. reported the use of nanolitre-sized droplets as an effective tool in coupling separations in both time and space. Using a microfluidic droplet connector, chemically separated components could be segmented into nanolitre droplets, and after oil filtering and droplet merging, the contents could be loaded into a second dimension for comprehensive separation (61).

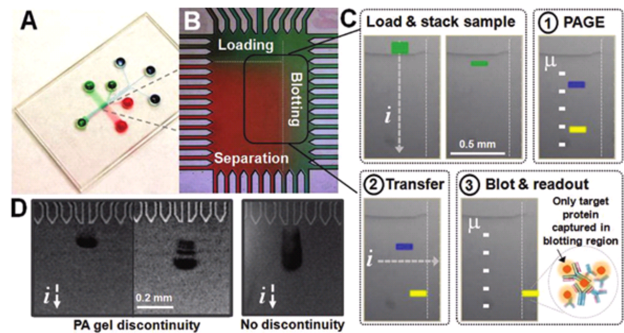

Fig. 3. Fully integrated protein immunoblotting using polyacrylamide gel photopatterning in a 2D microfluidic geometry. Bright field images of: (A) glass device with patterned PA gels and (B) magnified chamber with photopatterned gels for sample loading (blue), PAGE separation (red) and transfer to antibody functionalized blotting region (green). (C) The automated multistage assay protocol overlaid on micrographs on the microdevice. (D) Large-to-small pore size discontinuity at PAGE start yielding size-based PSA sample stacking (inverted grayscale). μ is mobility; "i" indicates direction of electrical current flow (53). Reproduced with permission from (53). Copyright 2010 American Chemical Society.

MICROFLUIDICS IN REAL-WORLD APPLICATIONS

As has been seen, microfluidic technology has great potential in many of biochemical and molecular biological applications. The high analytical efficiencies, fast processing times, reduced consumption of expensive biological reagents and portability make it suitable for a range of applications including diagnostics (46, 52, 62-70), protein and cell-based high throughput drug screening (26, 27, 71, 72) and biomolecule production through directed evolution (20). For example, microfluidic point-of-care diagnostic systems have gained much attention in recent years and proof of concept microfluidic diagnostic devices based on PCR (64, 65), DNA/microRNA profiling (66, 67), immunoassay (52, 70), and protein profiling (69) have all been demonstrated. Soh and Colleagues developed a microfluidic device for genetic analysis of the H1N1 influenza virus from throat swab samples (64). By integrating magnetic bead-based RT-PCR, denaturation, PCR amplification, generation of ssDNA, and target detection in a single device, the authors demonstrated detection of H1N1 at loads as low as 10 $TCID_{50}$ (4 orders of magnitude below the clinical titer for this virus). Also, Heath and co-workers demonstrated integrated barcode chips for rapid, multiplexed analysis of proteins using just microliter quantities of blood (69). In this study, multiple DNA-encoded antibody barcode arrays are patterned within the channel for protein measurement. Finally, as a first step towards disposable, stand-alone diagnostic devices for a range of fluorescence-based microfluidic assays, deMello and co-workers have pioneered the use of a polymer LEDs and photodiodes as excitation sources and photon detectors (73-76).

Such systems provide excellent detection limits and sensitivities whilst having small-footprints and being extremely cheap to fabricate and integrate with microfluidic systems (75).

CONCLUSION

This review has given a focussed snapshot of some recent advances in microfluidic technology for biochemistry and molecular biology. It can be seen that a diversity of analytical operations can be translated into chip-based formats with significant improvements in performance accompanying miniaturisation. For example, applications such as directed evolution (20), 2D immunoblotting (53), and diagnostic devices (64) exhibit fundamental improvements when transferred to planar chip formats, although it should be noted that interfacing with the macro-world is still a non-trivial task in most situations.

Since genomic and post-genomic sciences provide multiple challenges in terms of system complexity and the sheer volume of information that should be extracted from an analytical measurement, it will be necessary to develop novel and high-throughput microfluidic tools. It is expected that segmented-flow microfluidics will play a large part in allowing large-scale experimentation on such systems, however recent developments in chamber or surface based arrays (77) indicate new and powerful alternatives. Finally, it is noted that although microfluidics is still primarily used in academic laboratories, optimization of on-chip operations, integration of functional components, and interconnection between the device and the end user are providing more robust tools for commercial implementation. Several promising 'Killer applications' are already emerging, and microfluidic systems are likely to continue to impact the fields of biochemistry and molecular biology in the foreseeable future.

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REFERENCES

1. Hong, J., Edel, J. and deMello, A. J. (2009) Micro- and nanofluidic systems for high-throughput biological screening. *Drug Discov. Today* **14**, 134-146.
2. Liu, P. and Mathies, R. A. (2009) Integrated microfluidic systems for high-performance genetic analysis. *Trends Biotechnol.* **27**, 572-581.
3. deMello, A. J. (2006) Control and detection of chemical reactions in microfluidic systems. *Nature* **442**, 394-402.
4. Whitesides, G. M. (2006) The origins and the future of microfluidics. *Nature* **442**, 368-373.
5. Liu, K. and Fan, Z. H. (2011) Thermoplastic microfluidic devices and their applications in protein and DNA analysis. *Analyst.* **136**, 1288-1297.
6. Kartalov, E. P. and Quake, S. R. (2004) Microfluidic device reads up to four consecutive base pairs in DNA sequencing-by-synthesis. *Nucl. Acids Res.* **32**, 2873-2879.
7. Fredlake, C. P., Hert, D. G., Kan, C. -W., Chiesl, T. N., Root, B. E., Forster, R. E. and Barron, A. E. (2008) Ultrafast DNA sequencing on a microchip by a hybrid separation mechanism that gives 600 bases in 6.5 minutes. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 476-481.
8. Blazej, R. G., Kumaresan, P., Cronler, S. A. and Mathies, R. A. (2007) Inline injection microdevice for attomole-scale sanger DNA sequencing. *Anal. Chem.* **79**, 4499-4506.
9. Blazej, R. G., Kumaresan, P. and Mathies, R. A. (2006) Microfabricated bioprocessor for integrated nanoliter-scale sanger DNA sequencing. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 7240-7245.
10. Aborn, J. H., El-Difrawy, S. A., Novotny, M., Gismondi, E. A., Lam, R., Matsudaira, P., Mckenna, B. K., O'Neil, T., Streechon, P. and Ehrlich, D. J. (2005) A 768-lane microfabricated system for high-throughput DNA sequencing. *Lab. Chip.* **5**, 669-674.
11. Kumagai, H., Utsunomiya, S., Nakamura, S., Yamamoto, R., Harada, A., Kaji, T., Hazama, M., Ohashi, T., Inami, A., Ikegami, T., Miyamoto, K., Endo, N., Yoshimi, K., Toyoda, A. and Hattori, M. (2008) Large-scale microfabricated channel plates for high-throughput, fully automated DNA sequencing. *Electrophoresis* **29**, 4723-4732.
12. Novak, R., Zeng, Y., Shuga, J., Venugopalan, G., Fletcher, D. A., Smith, M. T. and Mathies, R. A. (2011) Single-cell multiplex gene detection and sequencing with microfluidically generated agarose emulsions. *Angew. Chem. Int. Ed.* **50**, 390-395.
13. Kong, D. S., Carr, P. A., Chen, L., Zhang, S. and Jacobson, J. M. (2007) Parallel gene synthesis in a microfluidic device. *Nucl. Acids Res.* **35**, e61.
14. Lee, C.-C., Snyder, T. M. and Quake, S. R. (2010) A microfluidic oligonucleotide synthesizer. *Nucl. Acids Res.* **38**, 2514-2521.
15. Kosuri, S., Eroshenko, N., LeProust, E. M., Super, M., Way, J., Li, J. B. and Church, G. M. (2010) Scalable gene synthesis by selective amplification of DNA pools from high fidelity microchips. *Nat. Biotechnol.* **28**, 1295-1301.
16. Schaerli, Y., Wootton, R. C., Robinson, T., Stein, V., Dunsby, C., Neil, M. A. A., French, P. M. W., deMello, A. J., Abell, C. and Hollfelder, F. (2009) Continuous-flow polymerase chain reaction of single-copy DNA in microfluidic microdroplets. *Anal. Chem.* **81**, 302-306.
17. Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. and Erlich, H. (1986) Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harbor Symp. Quant. Biol.,* **51**, 263-273.
18. Kopp, M. U., de Mello, A. J. and Manz, A. A. (1998) Chemical amplification: continuous-flow PCR on a chip. *Science* **280**, 1046-1048.
19. Tewhey, R., Warner, J. B., Nakano, M., Libby, B., Medkova, M., David, P. H., Kotsopoulos, S. K., Samuels, M. L., Hutchison, J. B., Larson, J. W., Topol, E. J., Weiner, M. P., Harismendy, O., Olson, J., Link, D. R. and Frazer, K. A. (2009) Microdroplet-based PCR enrichment for large scale targeted sequencing. *Nat. Biotechnol.* **27**, 1025-1034.
20. Agresti, J. J., Antipov, E., Abate, A. R., Ahn, K., Rowat, A. C., Baret, J. C., Marquez, M., Klibanov, A. M., Griffiths, A. D. and

Weitz, D. A. (2010) Ultrahigh-throughput screening in drop-based microfluidics for directed evolution. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 4004-4009.
21. Lion, N., Rohner, T. C., Dayon, L., Arnaud, I. L., Damoc, E., Youhnovski, N., Wu, Z.-Y., Roussel, C., Josserand, J., Jensen, H., Rossier, J. S., Przybylski, M. and Girault, H. H. (2003) Microfluidic systems in proteomics. *Electrophoresis* **24**, 3533-3562.
22. Zheng, B., Roach, L. S. and Ismagilov, R. F. (2003) Screening of protein crystallization conditions on a microfluidic chip using nanoliter-size droplets. *J. Am. Chem. Soc.* **125**, 11170-11171.
23. Zheng, B., Tice, J. D., Roach, L. S. and Ismagilov, R. F. (2004) A droplet based, composite PDMS/Glass capillary microfluidic system for evaluating protein crystallization conditions by microbatch and vapor-diffusion methods with on-chip x-ray diffraction. *Angew. Chem. Int. Ed.* **43**, 2508-2511.
24. Gerdts, C. J., Tereshko, V., Yadav, M. K., Dementieva, I., Collart, F., Joachimiak, A., Stevens, R. C., Kuhn, P., Kossiakoff, A. and Ismagilov, R. F. (2006) Time-controlled microfluidic seeding in nl-volume droplets to separate nucleation and growth stages of protein crystallization. *Angew. Chem. Int. Ed.* **45**, 8156-8160.
25. Kreutz, J. E., Li, L., Roach, L. S., Hatakeyama, T. and Ismagilov, R. F. (2009) Laterally mobile, functionalized self-assembled monolayers at the fluorous aqueous interface in a plug based microfluidic system; characterization and testing with membrane protein crystallization. *J. Am. Chem. Soc.* **131**, 6042-6043.
26. Srisa-Art, M., Kang, D., Hong, J., Park, H., Leatherbarrow, R. J., Edel, J. B., Chang, S. and deMello, A. J. (2009) Analysis of protein-protein interactions by using droplet based microfluidics. *Chembiochem.* **10**, 1605-1611.
27. Moorthy, J., Burgess, R., Yethiraj, A. and Beebe, D. J. (2007) Microfluidic based platform for characterization of protein interactions in hydrogel nanoenvironments. *Anal. Chem.* **79**, 5322-5327.
28. Gerber, D., Maerkl, S. J. and Quake, S. R. (2009) An *in vitro* microfluidic approach to generating protein-interaction networks. *Nat. Methods* **6**, 71-74.
29. He, M. and Herr, A. E. (2009) Microfluidic polyacrylamide gel electrophoresis with in situ immunoblotting for native protein analysis. *Anal. Chem.* **81**, 8177-8184.
30. Sikanen, T., Tuomikoski, S., Ketola, R. A., Kostiainen, R., Franssila, S. and Kotiaho, T. (2007) Fully microfabricated and integrated SU-8-based capillary electrophoresis-electrospray ionization microchips for mass spectrometry. *Anal. Chem.* **79**, 9135-9144.
31. Zheng, B., Gerdts, C. J. and Ismagilov, R. F. (2005) Using nanoliter plugs in microfluidics to facilitate and understand protein crystallization. *Curr. Opin. Struct. Biol.* **15**, 548-555.
32. Zheng, B., Roach, L. S. and Ismagilov, R. F. (2003) Screening of protein crystallization conditions on a microfluidic chip using nanoliter-sized droplets. *J. Am. Chem. Soc.* **125**, 11170-11171.
33. Meyvantsson, I. and Beebe, D. J. (2008) Cell culture models in microfluidic systems. *Annu. Rev. Anal. Chem.* **1**, 423-449.
34. El-Ali, J., Sorger, P. K. and Jensen, K. F. (2006) Cells on chips. *Nature* **442**, 403-411.
35. Kim, L., Toh, Y.-C., Voldman, J. and Yu, H. (2007) A practical guide to microfluidic perfusion culture of adherent mammalian cells. *Lab. Chip.* **7**, 681-694.
36. Goto, M., Sato, K., Murakami, A., Tokeshi, M. and Kitamori, T. (2005) Development of a microchip-based bioassay system using cultured cells. *Anal. Chem.* **77**, 2125-2131.
37. Urbanski, J. P., Johnson, M. T., Craig, D. D., Potter, D. L., Gardner, D. K. and Thorsen, T. (2008) Noninvasive metabolic profiling using microfluidics for analysis of single preimplantation embryos. *Anal. Chem.* **80**, 6500-6507.
38. Clark, A. M., Sousa, K. M., Jennings, C., MacDougald, O. A. and Kennedy, R. T. (2009) Continuous-flow enzyme assay on a microfluidic chip for monitoring glycerol secretion from cultured adipocytes. *Anal. Chem.* **81**, 2350-2356.
39. Huebner, A., Olguin, L. F., Bratton, D., Whyte, G., Huck, W. T. S., deMello, A. J., Edel, J. B., Abell, C. and Hollfelder, F. (2008) Development of quantitative cell-based enzyme assays in microdroplets. *Anal. Chem.* **80**, 3890-3896.
40. Shim, J.-U., Olguin, L. F., Whyte, G., Scott, D., Babtie, A., Abell, C., Huck, W. T. S. and Hollfelder, F. (2009) Simultaneous determination of gene expression and enzymatic activity in individual bacterial cells in microdroplet compartments. *J. Am. Chem. Soc.* **131**, 15251-15256.
41. Baret, J. C., Ryckelynck, M., El-Harrak, A., Frenz, L., Rick, C., Samuels, M. L., Hutchison, J. B., Agresti, J. J., Link, D. R., Weitz, D. A. and Griffiths, A. D. (2009) Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity. *Lab. Chip.* **9**, 1850-1858.
42. Ng, A. H. C., Uddayasankar, U. and Wheeler, A. R. (2010) Immunoassays in microfluidic systems. *Anal. Bioanal. Chem.* **397**, 991-1007.
43. Feldman, H. C., Sigurdson, M. and Meinhart, C. D. (2007) AC electrothermal enhancement of heterogeneous assays in microfluidics. *Lab. Chip.* **7**, 1553-1559.
44. Delamarche, E., Bernard, A., Schimid, H., Michel, B. and Biebuyck, H. (1997) Patterned delivery of immunoglobulins to surfaces using microfluidic networks. *Science* **276**, 779-781.
45. Sato, K., Tokeshi, M., Odake, T., Kimura, H., Ooi, T., Nakao, M. and Kitamori, T. (2000) Integration of an immunosorbent assay system: analysis of secretory human immunoglobulin a on polystyrene beads in a microchip. *Anal. Chem.* **72**, 1144-1147.
46. Sato, K., Tokeshi, M., Kimura, H. and Kitamori, T. (2001) Determination of carcinoembryonic antigen in human sera by integrated bead-bed immuoassay in a microchip for cancer diagnosis. *Anal. Chem.* **73**, 1213-1218.
47. Sato, K., Yamanaka, M., Takahashi, H., Tokeshi, M., Kimura, H. and Kitamori, T. (2002) Microchip-based immunoassay system with branching multichannels for simultaneous determination of interferon gamma. *Electrophoresis* **23**, 734-739.
48. Sato, K., Yamanaka, M., Hagino, T., Tokeshi, M., Kimura, H. and Kitamori, T. (2004) Microchip-based enzyme-linked immunosorbent assay (microELISA) system with thermal lens detection. *Lab. Chip.* **4**, 570-575.
49. Yang, Y., Nam, S., Lee, N., Kim, Y. and Park, S. (2008) Superporous agarose beads as a solid support for microfluidic immunoassay. *Ultra-microscopy* **108**, 1384-1389.
50. Cheng, S. B., Skinner, C., Taylor, J., Attiya, S., Lee, W. E., Picelli, G. and Harrison, D. J. (2001) Development of a mul-

ti-channel microfluidic analysis system employing affinity capillary electrophoresis for immunoassay. *Anal. Chem.* **73**, 1472-1479.
51. Qiu, C. X. and Harrison, D. J. (2001) Integrated self-calibration via electrokinetic solvent proportioning for microfluidic immunoassays. *Electrophoresis* **22**, 3949-3958.
52. Herr, A. E., Hatch, A. V., Throckmorton, D. J., Tran, H. M., Brennan, J. S., Giannobile, W. V. and Singh, A. K. (2007) Microfluidic immunoassays as rapid saliva-based clinical diagnostics. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 5268-5273.
53. He, M. and Herr, A. E. (2010) Polyacrylamide gel photopatterning enables automated protein immunoblotting in a two-dimensional microdevice. *J. Am. Chem. Soc.* **132**, 2512-2513.
54. Kenyon, S. M., Meighan, M. M. and Hayes, M. A. (2011) Recent developments in electrophoretic separations on microfluidic devices. *Electrophoresis* **32**, 482-493.
55. Tran, N. T., Ayed, I., Pallandre, A. and Taverna, M. (2010) Recent innovations in protein separation on microchips by electrophoretic methods: an update. *Electrophoresis* **31**, 147-173.
56. Fonslow, B. R. and Bowser, M. T. (2008) Fast electrophoretic separation optimization using gradient micro free-flow electrophoresis. *Anal. Chem.* **80**, 3182-3189.
57. Kostal, V., Fonslow, B. R., Arriaga, E. A. and Bowser, M. T. (2009) Fast determination of mitochondria electrophoretic mobility using micro free-flow electrophoresis. *Anal. Chem.* **81**, 9267-9273.
58. Turgeon, R. T. and Bowser, M. T. (2009) Improving sensitivity in micro-free flow electrophoresis using signal averaging. *Electrophoresis* **30**, 1342-1348.
59. Zalewski, D. R., Kohleyer, D., Schlautmann, S. and Gardeniers, H. J. G. E. (2008) Synchronized, continuous-flow zone electrophoresis. *Anal. Chem.* **80**, 6228-6234.
60. He, M. and Herr, A. E. (2010) Automated microfluidic protein immunoblotting. *Nat. Protoc.* **5**, 1844-1856.
61. Niu, X. Z., Zhang, B., Marszalek, R. T., Ces, O., Edel, J. B., Klug, D. R. and deMello, A. J. (2009) Droplet-based compartmentalization of chemically separated components in two-dimensional separations. *Chem. Comm.,* 6159-6161.
62. Kraytsberg, Y. and Khrapko, K. (2005) Single-molecule PCR: an artifact-free PCR approach for the analysis of somatic mutations. *Expert Rev. Mol. Diagn.* **5**, 809-815.
63. Gervais, L. and Delamarche, E. (2009) Toward one-step point-of-care immunodiagnostics using capillary-driven microfluidics and PDMS substrates. *Lab. Chip.* **9**, 3330-3337.
64. Ferguson, B. S., Buchsbaum, S. F., Wu, T. -T., Hsieh, K., Xiao, Y., Sun, R. and Soh, H. T. (2011) Genetic analysis of H1N1 influenza virus from throat swab samples in a microfluidic system for point-of-care diagnostics. *J. Am. Chem. Soc.* **133**, 9129-9135.
65. Park, S., Zhang, Y., Lin, S., Wang, T. -H. and Yang, S. (2011) Advances in microfluidic PCR for point-of-care infectious disease diagnostics. *Biotechnol. Adv.* **29**, 830-839.
66. Yeung, S. H. I., Seo, T. S., Crouse, C. A., Greenspoon, S. A., Chiesl, T. N., Ban, J. D. and Mathies, R. A. (2008) Fluorescence energy transfer-labeled primers for high-performance forensic DNA profiling. *Electrophoresis* **29**, 2251-2259.
67. Anglicheau, D., Sharma, V. K., Ding, R., Hummel, A., Snopkowski, C., Dadhania, D., Seshan, S. V. and Suthanthiran, M. (2009) MicroRNA expression profiles predictive of human renal allograft status. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5330-5335.
68. Koirala, D., Yu, Z., Dhakal, S. and Mao, H. (2011) Detection of single nucleotide polymorphism using tension-dependent stochastic behavior of a single-molecule template. *J. Am. Chem. Soc.* **133**, 9988-9991.
69. Fan, R., Vermesh, O., Srivastava, A., Yen, B. K. H., Qin, L., Ahmad, H., Kwong, G. A., Liu, C.-C., Gould, J., Hood, L. and Heath, J. R. (2008) Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. *Nat. Biotechnol.* **26**, 1373-1378.
70. Henares, T. G., Mizutani, F. and Hisamoto, H. (2008) Current development in microfluidic immunosensing chip. *Anal. Chim. Acta.* **611**, 17-30.
71. Granieri, L., Baret, J. C., Griffiths, A. D. and Merten, C. A. (2010) High throughput screening of enzymes by retroviral display using droplet based microfluidics. *Chemistry & Biology* **17**, 229-235.
72. Brouzes, E., Medkova, M., Savenelli, N., Marran, D., Twardowski, M., Hutchison, J. B., Rothberg, J. M., Link, D. R., Perrimon, N. and Samuels, M. L. (2009) Droplet microfluidic technology for single cell high throughput screening. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14195-14200.
73. Wang, X., Amatatongchai, M., Nacapricha, D., Hofmann, O., de Mello, J. C., Bradley, D. D. C. and de Mello, A. J. (2009) Thin-film organic photodiodes for integrated on-chip chemiluminescence detection-application to antioxidant capacity screening. *Sensor Actuat. B-Chem.* **140**, 643-648.
74. Yamazaki, M., Hofmann, O., Ryu, G., Xiaoe, L., Lee, T. K. and deMello, A. J. (2011) Non-emissive colour filters for fluorescence detection. *Lab. Chip.* **11**, 1228-1233.
75. Ryu, G., Huang, J., Hofmann, O., Walshe, C. A., Sze, J. Y. Y., McClean, G. D., Mosley, A., Rattle, S. J., deMello, J. C., deMello, A. J. and Bradley, D. D. C. (2011) Highly sensitive fluorescence detection system for microfluidic lab-on-a-chip. *Lab. Chip.* **11**, 1664-1670.
76. Hofmann, O., Wang, X., deMello, J. C., Bradley, D. D. C. and deMello, A. J. (2005) Towards microalbuminuria determination on a disposable diagnostic microchip with integrated fluorescence detection based on thin-film organic light emitting diodes. *Lab. Chip.* **5**, 863-868.
77. Heyries, K. A., Tropini, C., VansInsberghe, M., Doolin, C., Petriv, O. I., Singhal, A., Leung, K., Hughesman, C. B. and Hansen, C. L. (2011) Megapixel digital PCR. *Nat. Methods* **8**, 649-651.