Microfluidic Systems

Integrated Microfluidic Systems**

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Keywords:
analytical methods · enzymes · microfluidics · microreactors · protein structures

Microfluidic systems use networks of channels thinner than a human hair to manipulate nanoliter volumes of reagents. The goal of microfluidics is challenging: to integrate, on a chip smaller than a credit card, all operations normally performed in a chemical or medical laboratory. Synthesis, purification, analysis, and diagnostics would be performed by such a "lab on a chip" rapidly, economically, and with minute volumes of samples. This scale-down approach is inspired by, and often compared to, the success of the miniaturization in the computer industry, namely, the miniaturization and integration of thousands of transistors on a silicon chip that has led to a rapid increase in performance and decrease in the cost of computers. This highlight describes two significant steps towards these ambitious goals.

Multistep Catalytic Reactions using Microbeads

Seong and Crooks[1] have reported a microfluidic system that relies on microscopic beads trapped in microfluidic channels to perform sequential catalytic chemical reactions. It is remarkable that the microbeads serve two functions simultaneously: they induce rapid mixing of the reagents (Figure 1) and serve as the support for immobilized enzymatic catalysts (Figure 2).

Mixing solutions in microfluidic channels is difficult—two streams, injected into a microchannel, co-flow with slow mixing only through diffusion. Efficient mixing occurs when these streams are split into thin substreams ("laminae") and then recombined in such a way that the laminae of the two different fluids come in contact. Mixing is accelerated because diffusion through the thin laminae is fast, and because the laminae are in contact over a large total surface area. This splitting and recombination does not occur in simple geometries in a steady laminar flow, but it has been induced using several methods. For example, three-dimensional microfabrication may be used to induce multilaminar mixing directly[2] or to induce chaotic advection,[3] which repetitively splits and recombines the streams to achieve thinner and thinner laminae.[4,5] Alternatively, time may be used as the third dimension,[3] and chaotic advection may be induced in unsteady, time-periodic flows in droplets moving through channels fabricated by conventional two-dimensional methods.[6]

Seong and Crooks reported the remarkable observation that such splitting and recombination (distributive mixing) can be achieved simply by flowing solutions through beads packed inside a microfluidic channel (Figure 1); these beads are only a few times (ca. 10) smaller than the channel. The flow around a single large bead in a channel can be modeled easily. One would expect that such a bead would not induce significant mixing. The flow through a channel containing thousands of perfectly packed beads can be modeled by mean-field approximations. One would expect that a large number of very small beads perfectly packed in a channel would not induce such mixing either. The intermediate regime, with just a few beads across the width of the channel, may be especially challenging from a theoretical point of view because the flow patterns would strongly depend on packing defects that are likely to be present when rectangular channels are packed with spherical beads of inter-

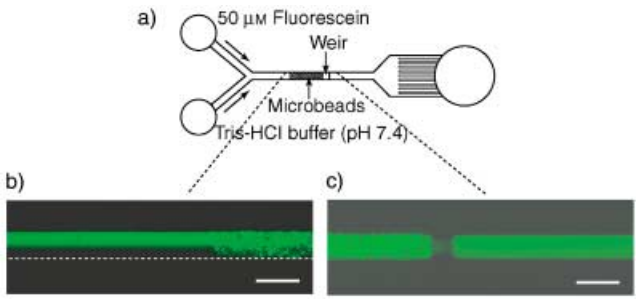

Figure 1. The mixing of two laminar streams by microbeads trapped inside a microfluidic channel.[1] a) A schematic drawing of the microfluidic device. b) A fluorescent microphotograph of distinct laminar streams of fluorescein and buffer entering the bead-packed region of the microchannel. c) A fluorescent microphotograph illustrating that the two streams are completely mixed upon exiting the bead-packed region of the microchannel. Scale bars are 200 μm. Tris = tris(hydroxymethyl)aminomethane. Reprinted (in part) with permission from the American Chemical Society. Copyright 2002.

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[**] Original figures were generously provided by Prof. Richard Crooks and by Prof. Stephen Quake. Support by ONR is gratefully acknowledged.

  DOI: 10.1002/anie.200301660

mediate size (especially if the beads are not uniform). An interesting possibility is that a three-dimensional network of these defects could effectively split and recombine the flows; presumably, mixing could be further enhanced by inducing and controlling these defects. These results are certain to stimulate exciting theoretical and experimental work, and it is rewarding to see new developments in chemistry posing new questions for physics.

Microbeads bearing immobilized enzymes[7] and heterogeneous catalysts[8] can be used to carry out reactions efficiently because they provide the high surface-to-volume ratio required for heterogeneous reactions.[8] An attractive feature of the system described by Seong and Crooks is the ability to carry out multistep transformations. To demonstrate this capability, they created microfluidic channels with two regions packed with trapped beads: the first with immobilized glucose oxidase and the second with immobilized horseradish peroxidase (HRP; Figure 2). The flow of a solution of glucose through the channels resulted in the catalytic oxidation of glucose by glucose oxidase and the generation of H_2O_2. A solution of the nonfluorescent dye amplex red was added downstream from the first pack of microbeads. Horseradish peroxidase immobilized on the second pack of beads used the H_2O_2 liberated in the first reaction to catalytically oxidize this dye and produce red fluorescent resorufin. This system will open many exciting opportunities in biochemical analysis and synthesis that involves multistep catalytic reactions.

Microfluidic Large-Scale Integration

The development reported by Quake and co-workers[9] provides a strong corroboration to the analogy between the miniaturization of computers systems and the miniaturization of microanalytical systems. Their work presented a solution for the large-scale integration (LSI) problem of microfluidic networks. In computer terminology, the term LSI refers to the ability to create large networks of transistors on a computer chip. The biggest problem in operating a computer chip with an array of millions of transistors is addressability, that is, being able to control (address) all the transistors using a minimal number of electrical connections to the outside world. The problem is similar in operating a microfluidic system containing thousands of microscopic reaction volumes: filling these reaction volumes with reagents using a minimal number of channels that have to be controlled.

The first problem solved by Quake and co-workers is microfluidic multiplexing, which is the ability to control flow through a large number (F) of flow channels using only a small number (C) of control channels. The fundamental form of control is on/off switching of the flow in the flow channel using a valve. Small systems can be easily controlled by introducing a valve in each flow channel. However, the number of the control elements equals the number of flow channels ($C = F$) and becomes prohibitively large as the size of the system is increased. Quake and co-workers solved this problem by using microfluidic multiplexing, an elegant analogy to the multiplexors used in computer chips. These systems rely on the ability to fabricate three-dimensional structures in poly(dimethylsiloxane) (PDMS) and the ability to create valves at crossings of microchannels by using the elastomeric properties of PDMS.[10] The principle of multiplexing is simple and is illustrated for eight flow channels controlled by three pairs of control channels (Figure 3). One control channel within a pair operates valves that can close half (4 out of 8) of the flow channels, while the other control channel closes the remaining half of the flow channels. In the same way as any number from zero to seven can be represented by a three-bit binary code, any of the eight channels can be left open (addressed) using three pairs of

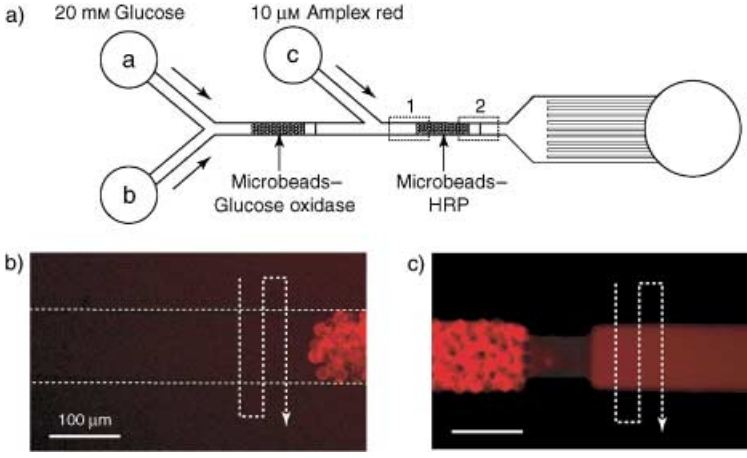

Figure 2. Two-step chemical transformation using catalyst-bearing microbeads in a microfluidic channel.[1] a) A schematic illustration of the microfluidic device. b) A fluorescent microphotograph of the fluid entering the second bead-packed region (rectangle 1 in a). c) A fluorescent microphotograph of the bright fluorescence of the fluid exiting the second bead-packed region (rectangle 2 in a). Fluorescent resorufin was formed by enzyme-catalyzed oxidation of amplex red by H_2O_2, which is produced in turn by glucose oxidase immobilized in the first bead-packed region. Reprinted (in part) with permission from the American Chemical Society. Copyright 2002.

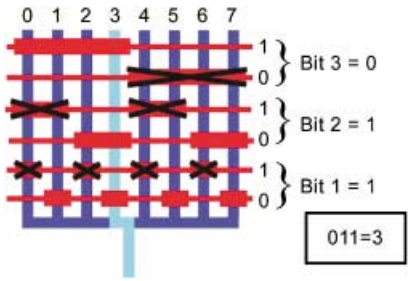

Figure 3. A schematic diagram illustrating the principle of microfluidic multiplexing.[9] Fluid flow through the eight vertical "flow channels" can be controlled using three pairs of horizontal "control channels". The wide sections of the control channels correspond to valves that can close off flow channels. Each pair of control channels may be described as one binary bit, and each binary three-bit number corresponds to the opening of one of the eight flow channels. Excerpted with permission from the American Association for the Advancement of Science. Copyright 2002.

control channels. Since $C = 2\log_2 F$, the decrease in the number of control elements for small networks is modest, and six control channels are required for eight flow channels. For larger networks, the decrease is substantial—only 20 control channels are needed for 1024 flow channels.

To demonstrate the functionality of their system an array of 256 individually addressable microreactors was created. Figure 4 demonstrates that each microreactor can be filled with two different solutions, allowed to mix and react, and then the products can be selectively isolated. This system was then used to perform a high-throughput detection of single bacterial cells expressing recombinant cytochrome c peroxidase.

In a subsequent paper,[11] Quake and co-workers have shown how this method may be applied to the screening of conditions that induce the crystallization of proteins, a problem which is both difficult and important. Screening is very common in protein crystallization, but involves either expensive automation or extensive manual labor. In addition, prohibitively large volumes of concentrated solutions of proteins are required for screening. Microfluidic systems have the potential to solve all of these problems at once: they are inexpensive, could be automated, and consume minimal amounts of proteins. For example, this microfluidic chip performed 144 trials in parallel and each of the trials required only 10 nL of the protein solution. Manual dispensing would have required approximately 100 times larger amounts of proteins. The solutions of the protein and a precipitant were combined in each compartment of this chip (Figure 4) and crystals formed as the solutions mixed slowly by diffusion—the free-interface diffusion method. It is significant that prior to this microfluidic method, crystallization by the free-interface diffusion method had not been possible under Earth's gravity.

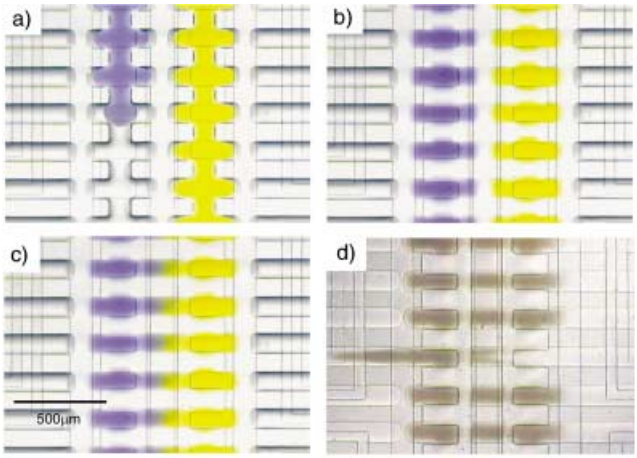

Figure 4. Complex manipulation of multiple solutions on a microfluidic chip using microfluidic multiplexing.[9] Hundreds of samples can be loaded (a), compartmentalized (b), allowed to mix and react (c), and then individual samples can be isolated (d) for further analysis or characterization. Reprinted with permission from the American Association for the Advancement of Science. Copyright 2002.

Conclusion and Outlook

Microfluidics is an exciting interdisciplinary field where chemistry, biology, physics, and engineering synergistically come together. The work discussed here clearly shows that the approach of miniaturization and integration is bearing fruit. Microfluidics is stimulating new chemistry—from the control of surface chemistry and development of new materials to the development of new analytical and synthetic methodologies that take full advantage of the new microscale technologies. It is especially exciting that microfluidics is also enabling chemistry to be carried out.

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