PDMS valves in DNA computers

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ABSTRACT

DNA computing is an interdisciplinary field accessing the possibility for the use of biomolecules, such as DNA, RNA and proteins, as a computational or control tool. Traditionally, DNA computers were thought to compete with electronic computers to solve, for example, NP-complete problems. However recently, there has been a focus shift to biomedical applications.
One form of DNA computing is performed in microfluidics. A network of microreactors decides the computational aspects and DNA is the tool for selection procedures. To control complex microflow systems like this, a series of pneumatic valves are used to control the flow direction, i.e. the information direction, and to contain DNA functionalised beads in the microreactors.

Keywords: DNA computing, beads, hybridization, PDMS valves

1. INTRODUCTION

The advantage of microfluidics is that it reduces the size of the experimental set-up, allowing an easier use and overview. But it also reduces the price and the amount of consumables used. We have applied the field of microfluidics to biomolecular computing, or better known as DNA computing [1].
Biomolecular computers are very promising because they are closely related to the biological world. Since the information is offered and processed in biomolecules, the result can be biomolecules as well. This means it could possibly facilitate drug design. Therefore biomolecular computers can have applications in biotechnology, such as medical diagnostics and drug lead-compound optimisation [2, 3]. Also, the research on operations with biomolecules give a better insight into biological systems, while the information processing and construction capabilities at molecular level give rise to new computing paradigms. The optimal computing solution is the integration of both silicon and molecular computers, using the conventional computer as a controller [4, 5].
Microfluidic networks are incorporated as an information carrier in a molecular computing scheme [6]. The channels are like the wires in electronic circuits, transporting information from one operator to another, an operator being a fluidic flip-flop, *i.e.* logical operator. The advantages of microfluidics are the small volumes (in the pico-liter range) of molecular reagents needed and the fast reaction rates due the rapid diffusions of molecule in small volumes. With fluidic valves, *e.g.* PDMS valves, and micro pumps the flow can be (re-)directed [7], while beads are good supports for capture probes [8].
In this paper, we introduce microfluidic logic operators, capture probes and simple fluidic valves that act as bead barriers.

2. LOGIC OPERATIONS

Selections are made by using capture probes (CP), utilizing short single stranded DNA, representing bits [6]. From a designed molecular library $\{S_i\}$, these CPs select longer single DNA strands containing information in the sequence of its base-pairs, representing words, *i.e.* sequences of bits (see Fig. 1). Hybridisation between the CP and information strand is a selection, a YES or NO, *i.e.* a logic operation.

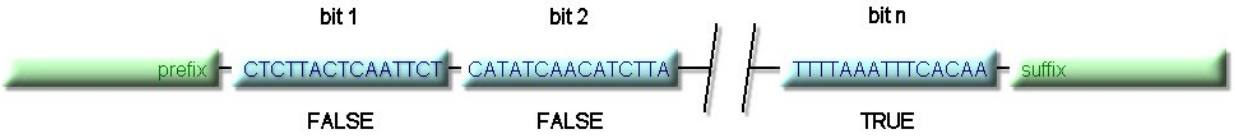


Figure 1. A word consists of a sequence of bits with value TRUE or FALSE, while each bit is coded by a short DNA-strand, typically 15 base-pairs.

Logic operators can be implemented by using positive selection, which retains the desired strand S_k from the sequence space $\{S_i\}$, or negative selection, which discards S_k in a particular logical operation. The operation ***a*** is a positive selection and ***¬a*** a negative selection procedure (Fig 2a). Placing two selectors in sequence will perform an AND operation, while two selectors in parallel will perform an OR operation (see Fig 2b) [6].

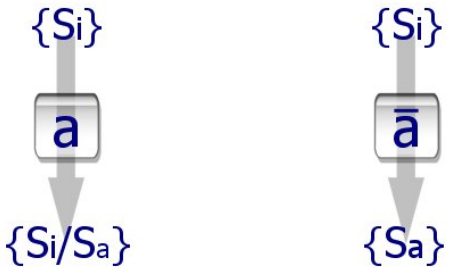


Figure 2a NOT operations on member a in a sequence space $\{S_i\}$ is made by a negative selection, while selection of member a (S_a) is made by a positive selection.

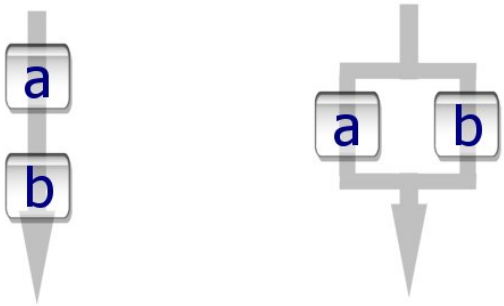


Figure 2b. AND operations can be made by having two selectors in sequence, while an OR operation is made by two selectors in parallel.

By making a combination of a number of selectors, it is possible to solve problems from the Boolean logic, which is the basic of any computational problem.
Experiments in microreactors have concluded [9] that a typical hybridisation time lies within 4 minutes, while hybridisations in test tubes can take 24 hours.
Figure 3 shows a typical hybridisation curve. Streptavidin functionalised beads (5.6 µm, Bangs Laboratories Inc., IN, USA) were coupled with biotinylated oligo $(dT)_{25}$ (from Integrated DNA Technologies Inc., IA, USA), *i.e.* a DNA sequence consisting of 25 thymines. The concentration of the input strand $(dA)_{25}$ was in a pM range. 10 µl of the solution and an intercalater YOYO-1 (Molecular Probes Inc., OR, USA) was pumped into the reactor with a flow rate of

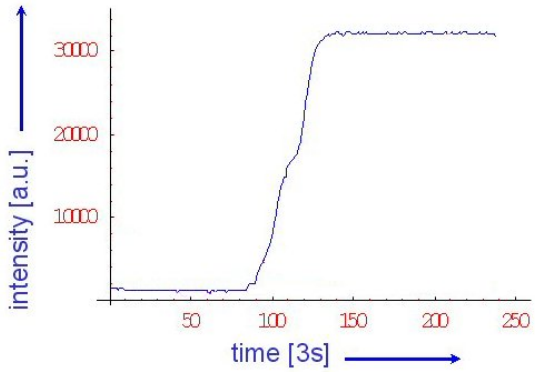


Figure 3. The hybridisation curves for a concentration of 0.5 pM of $(dA)_{25}$. The slope of the curves shows the dynamics of the hybridisation.

1 µl/min, which was placed under a fluorescence microscope. The fluorescence probe will only light up when hybridisation takes place, *i.e.* there is a double stranded DNA. Every 3 seconds an intensity measurement was taken with an analogue video camera and stored on disk. By using image processing techniques, the intensity of the signal could be determined. The analysis was performed in Mathematica (Wolfram Research Inc., IL, USA). From the slope of the curve the dynamics of the hybridisation can be determined.

3. BEAD BARRIERS

To do hybridisation, *i.e.* selection, functionalised beads are used. However, beads have to be somehow fixed in the microfluidic system. One way to trap beads in a certain location, or microreactor, is to use bead barriers [10]. Barriers are elevated sections in the microchannel which leaves a gap between the device cover and barrier which is smaller than the bead's diameter. It should be noted that somehow the beads have to be transported to the reactor. This can be done by adding a bead delivery channel (see Fig. 4) to the system which is connected to the reactor. The bead delivery channel has the same depth as the reactor, while the input and output channel form the barrier. However, once the beads are in it is not so easy to remove them from the reactor.

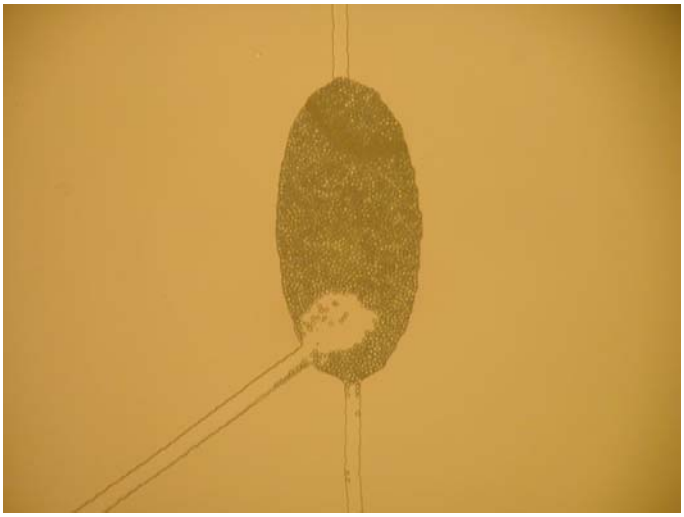


Figure 4. 5.6 µm beads in a reactor were the vertical in- and output channels (50 µm wide, 3 µm deep) function as bead barriers, while the diagonal bead delivery channel (100 µm wide, 10 µm deep) has the same depth as the reactor.

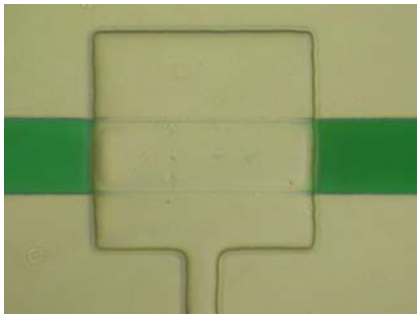


Figure 5. A fully closed pneumatic valve (300x300 µm square) over a 100 µm channel, effectively blocking the channel filled with green dye.

A more flexible method to trap beads is by using pneumatic valves to function as bead barriers in certain locations in a fluidic network. These pneumatic valves were fabricated on top of the flow channels, were made of PDMS (Polydimethylsiloxane, Sylgard 184, Dow-Corning, MI) [7] and consist of a very thin membrane (~40 µm). When pressure is applied, the membrane is pushed into the underlying channel, effectively blocking the flow (Fig. 5). The degree to which the channel is closed depends on the pressure applied, making this valve comparable to a potentiometer in electronics. By allowing the valve to close partially, beads were captured while the flow could continue, turning this device into a bead barrier (see Fig. 6).
One of the advantages is that the beads can be loaded through the same channel structure as the flow, so no alternative bead delivery channels are needed, while the valves are used to control the distribution of beads. Furthermore, the valve-system is a completely different circuit from the flow structure, which means there will be no interference with the flow due to the structure, as is the case with bead delivery channels. After the beads have been used, they can be flushed out by opening all the valves, thus making the structure reusable. The beads can then be processed for further analysed or discarded.

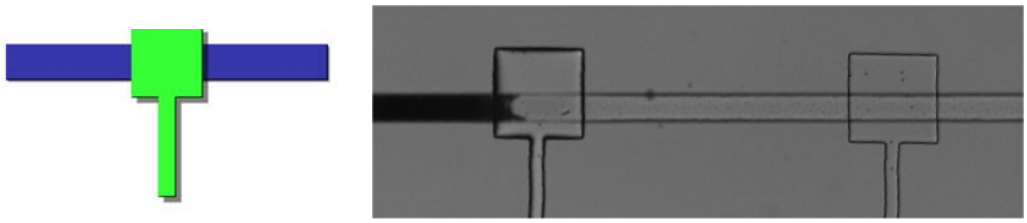
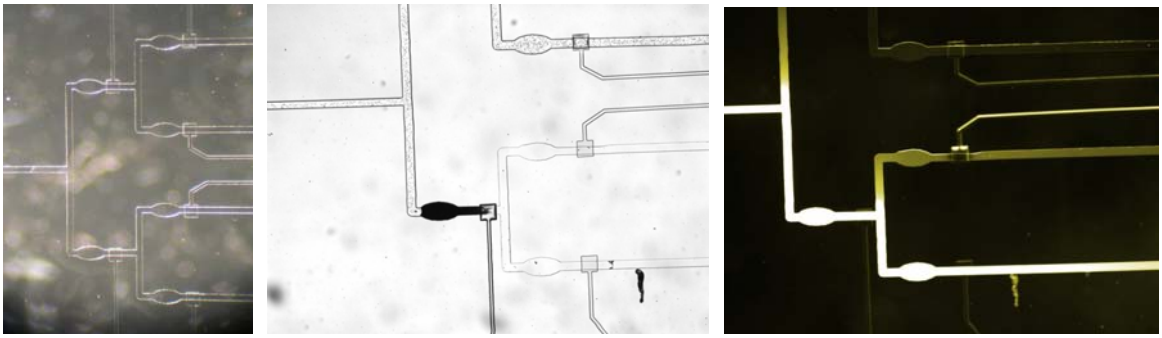


Figure 6. (a) a schematic representation of a valve. The dark gray channel is the solution flow channel, while the light gray channel is the pneumatic valve over the flow channel separated by a 40 µm membrane. (b) 5.6 µm beads (on the left side of the valve) were collected before a partially closed valve. The flow channel is 100 µm and the valve pad is 300x300 µm.

A flow structure was designed and manufactured incorporating 6 PDMS valves to form 6 reactor zones which could be filled up with beads (Fig. 7a). This microsystem consisted of 1 input and 4 outputs. The flows can be regulated by closing the PDMS valves. In this fashion it was possible to fill up each reactor by setting the valves in a certain configuration (Fig. 7b). Paths can be illuminated by using a fluorescence dye, such as Rhodamine 6G (Molecular Probes, OR, USA) and a monochrome light source (Fig. 7c).
This system can be used to set up a simple 2-bit OR. Going from bottom up in Fig. 6a, the layer with the first two reactors can be used as a selector for the 2-bit OR problem (hence the split after the input), by negative selection. The next layer, having 4 reactors, can be used to do detection with a positive selection step. The intensity levels measured in this layer can be compared and an assessment made. Results will be shown elsewhere.



A B C

Figure 7. (a) the reactor layout in PDMS, containg 6 valves after each of the 6 reactors. (b) a reactor being filled with beads. Notice that only where the valve is partially closed beads are trapped. (b) a fluorescence image of a flow path with Rhodamine 6G through the microsystem. By completely closing the valve, the flow direction can be regulated.

4. FABRICATION STEP

A master was fabricated by photolithographic patterning of a negative photoresist (SU-8 2007, Microlithography Chemical Corp. Newton, MA, USA) using high resolution printed transparencies (Pageworks, MA, USA). The features height was 10 µm, for both valves and channels. A pre-determined amount of PDMS (5:1) was then cast against the master with the channel structures and then degassed. To make the valve layer, degassed PDMS (30:1) was spin coated on the mask at 2000 rpm for 30 sec. resulting in a thin layer of ~40 µm. Both layers were cured for 20 min. at 80 ^{o}C, after which the channel layer was removed from the mask, aligned with the valve layer, brought in to contact and cured for another 4 hours.

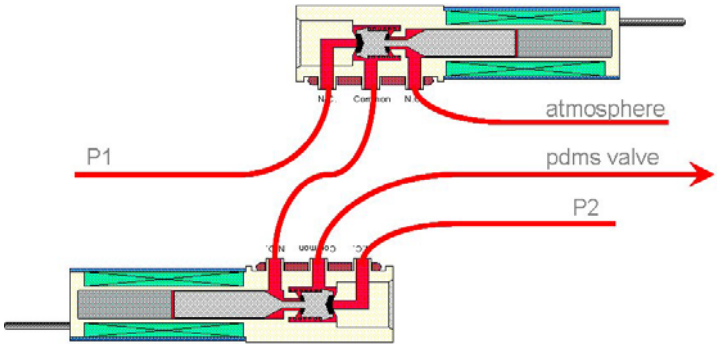
Inlets and outlets were made by punching holes (Technical Innovations Inc., TX, USA) through the PDMS. The structures were sealed irreversibly by plasma treatment of the PDMS mould and a 0.17 mm microscope coverslip (Fisher Scientific, PA, USA), after which they were brought into contact. Tubes were inserted into the inlets and connected to a syringe pump (Harvard Apparatus, MA, USA). To measure the hybridisation dynamics, the microfluidic system was placed under a fluorescence microscope (Nikon, USA).

5. CONTROL AND FLUIDIC INTERFACE

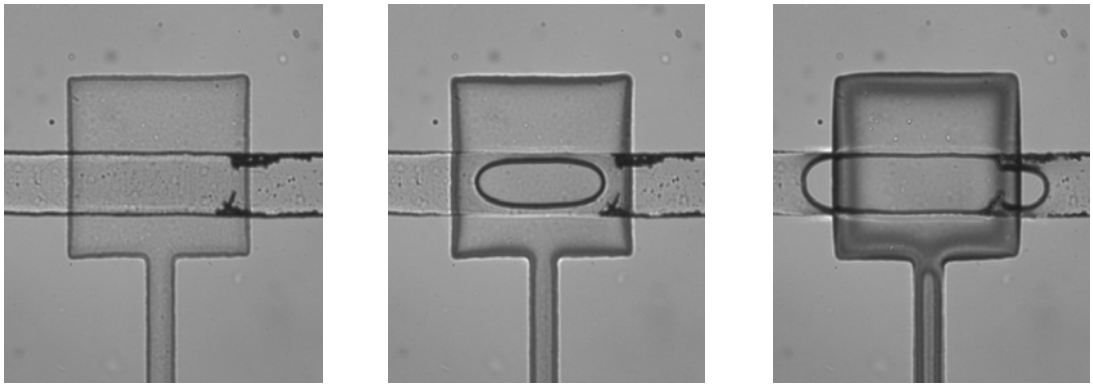
Microfluidic systems can be readily automated by using peripheral components to operate the pumps and PDMS valves. For example, the syringe pump used in this work (Harvard PHD 2000) can be fully controlled over RS-232. The PDMS valves were pneumatically controlled by a 3-way solenoid valve (Lee Company, CT, USA). By coupling two solenoid valves in series (Fig. 8a) it was possible to regulate the PDMS valve at three different pressure levels; high (30 psi), medium (15 psi) and low (atmosphere; Fig. 8b). The solenoid valves where controlled by a conventional computer running LabVIEW with a DAC-board (NI PCI-6533, National Instruments). Transistor switching boards (Comfile Technology, South Korea) where used to switch the 12V solenoids with a 5V TTL-signal. The pressured air was obtained from the laboratory's outlet and down regulated using mini pressure regulators.

The fluidic interfaces to the macro-world were made by inserting short metal tubing (New England Small Tube Corporation, NH, USA) into the holes punched into the PDMS. Tygon tubing (Fisher Scientific, PA, USA) with an inner diameter of 1/16" fitted tightly over the metal tubes, forming a perfect connection, even at relatively high pressures.

(a)



(b)



atmosphere 15 psi 30 psi

Figure 8. (a) By putting 2 bi-stable solenoid valves in series it is possible to apply 3 different pressures to the PDMS valves, where P_i is the pressure. (b) The pneumatic PDMS valve (300x300 µm square) over a 100 µm channel at atmosphere, 15 and 30 psi. As can be seen, at 15 psi the valve is not completely closed, allowing for a decreased flow, but retaining the beads.

6. CONCLUSION

The use of PDMS valves as bead barriers proves to be more flexible than previously proposed systems. It further shows that the integration of electronic control with microfluidic is essential for future progress not only in the molecular computing world, but in the biotechnology in general as well. It is easy to see that a system like this can be applied in areas of biotechnology, like in medical diagnostics.
It should further be noted that all logic operations can be performed with these microfluidic system. The end result of the selection has the DNA with the instruction set desired (the DNA is an instruction template).

7. ACKNOWLEDGEMENT

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REFERENCES

1. Adleman, L. M. (1994) Molecular computation of solutions to combinatorial problems. Science **266**, 1021-1024.
2. Benenson, Y., Gil, B., Ben-Dor, U., Adar, R. and Shapiro, E. (2004) An autonomous molecular computer for logical control of gene expression. Nature **429**, p423.
3. Lee, J-Y, van Noort, D., Tai Hyun Park and Zhang, B-T (2004) DNA computational medical diagnostics in microfluidics. Submitted to this conference
4. Suyama, A. (2002) Programmable DNA computer with application to mathematical and biological problems. Preliminary Proceedings, Eight International Meeting on DNA Based Computers, June 10-13, 2002, Japan, 91.
5. van Noort, D. (2004) A programmable molecular computer in microreactors. In press, LNCS.
6. van Noort, D., Gast, F.-U. and McCaskill, J. S. (2001) Computing in microreactors. LNCS **2340**, 33-45.
7. Unger, M. A., Chou, H.-P., Thorsen, T., Scherer, A. and Quake, S. R. (2000) Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography. Science **288**, 113-116.
8. Verpoorte, E. (2003) Beads and chips: new recipes for analysis. Lab Chip **3**, 60N-68N.
9. van Noort, D and Landweber, L. F. (2003) Towards a re-programmable DNA computer. In press, J. of Natural Computing.
10. McCaskill, J. S. (2001) Optically programming DNA computing in microflow reactors. Biosystems **59**, 125-138.