

Lab on a Chip

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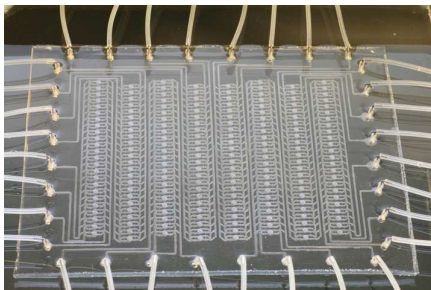
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We describe a concept of fluidic microprocessors based on components with decision-making ability, which actively process chemical information.



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Fluidic microchemomechanical integrated circuits processing chemical information

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The lab-on-a-chip (LOC) technology has been discussed to blossom into a major new technology fundamentally influencing the sciences of life and nature. From a systemic point of view however, microfluidics is still in its infancy. Here, we present the concept of a microfluidic central processing unit (CPU) which shows remarkable similarities to early electronic Von Neumann microprocessors. It combines both control and execution unit and, moreover, the complete power supply on a single chip and introduces the decision-making ability regarding chemical information into fluidic integrated circuits (ICs). As a consequence of this system concept, the ICs process chemical information completely self-controlled and energy self-sustaining. The ICs are fabricated by layer-by-layer deposition of several overlapping layers based on different intrinsically active polymers. As examples we present two microchips carrying out long-term monitoring of critical parameters by around-the-clock sampling.

Introduction

In the sciences of life and nature the importance of manipulating and processing liquid flows containing molecules and organisms is comparable to the importance of manipulating streams of electrons in microelectronic information technology (IT).¹⁻⁷ Fluidic integrated circuits (IC), the so-called labs-on-a-chip, could revolutionise the processing of liquid flows in a similar way as electronic ICs changed IT. Therefore, they are discussed as a major new technology of this century.^{5,7} A broad range of methods to manipulate liquids on a microchip have been developed over more than twenty years.⁸ However, in 2010 G. Whitesides noted disillusioned, that the expected revolution of the LOC technology has not yet happened.⁷ The reasons are currently discussed controversially. As a key to success several scientists recommend the intensification of solving commercially important problems.^{7,8} In our opinion, there are some strong arguments not to neglect basic research. We explain that by a comparison of the current LOC technology with the main factors of success of the early electronic microprocessors of the seventies from a systemic point of view.

Von Neumann computer. This is a traditional uniprocessor machine like a personal computer which processes both instruction and data stream sequentially.⁹ Such a device consists of an execution unit, control unit, memory, input/output (i/o) unit and a power supply (Fig. 1A). Control and execution unit are combined on a single integrated circuit, the microprocessor (central processing unit). The control unit handles the instructions and prepares them for the execution unit. Instructions are processed by the Von Neumann cycle, which consists of five steps (Fig. 1D).

The functionality and performance of microprocessors depend strongly on the number of components integrated into the IC. The success story of microelectronics started at the beginning of the seventies with the market launch of the first electronic pocket calculators which were – due to their low price – for the first time of interest for a broad public. Core of these devices were the first microprocessors, e.g. from Texas Instruments and Intel, which were large-scale integrated ICs consisting of several thousands of transistors.^{10,11} This high integration degree allowed to realise the complex functionality of a microprocessor on a single IC.

The set of electronic components consists of passive (e.g. resistors, capacitors, inductors) and active components such as transistors. These transistors are of particular interest, because they allow an electrical charge transfer if a defined threshold voltage is reached, that is, they perform decisions. This decision-making ability of transistors is the foundation of the logic data processing of microelectronics and thus the base of processing long program sequences. Today, computers solve complex calculations, simulations and other tasks of information processing which could not be mastered by man alone.

LOC system based on the concept of microelectro-mechanical systems (MEMS). The current LOC technology is almost exclusively based on the MEMS concept.^{8,12-14} The architecture of a MEMS-based LOC is shown in Fig. 1B. The fluidic IC includes the execution unit consisting of fluidic channel network and fluidic data storage (mostly not designed as separate unit, since usually the results of the fluidic operations remain directly in the channel network), whereas the complete control unit remains off-chip. Sometimes the IC contains parts of the i/o unit such as electrodes for the electrochemical analysis.⁸

Such a device processes instructions by the basic cycle shown

in Fig. 1E. A computer provides a defined sequence of electronic control signals, which have to be transformed by an electromechanical transducer (e.g. pneumatic controllers for micropneumatics, special electronics for electrokinetic and acoustic platforms as well as mechatronic disk drives)^{1,15-19} into mechanical or mechanically acting instructions. Chemical data are loaded from the fluidic data storages or the i/o unit. The actual mechanical execution of the instruction consists in the fluid transport with a given configuration of all valves for a defined time interval. Further processing of the chemical data occurs by chemical reactions, e.g. in reaction chambers. In order to simplify the description of the behavior of the chip, it is reasonable to group certain successions of elementary instructions into one complex instruction that executes a specific task.

The majority of fluidic ICs of lab-on-a-chip systems are small-scale (less than ten active components) or medium-scale integrated microchips with up to hundred active components, mostly microvalves and micropumps. Currently, only the micropneumatic platform technology gives access to large-scale integrated ICs with thousands of components,^{8,13,14} which is however, mainly focussed on the high parallelisation of simple tasks with short program sequences.

Furthermore, current MEMS based ICs lack components that are able to perform decisions based on chemical information (i.e. concentrations of chemical substances).^{8,14,20} The absent decision-making ability is the main reason for the incapability of current microfluidics to perform long, complex program sequences.

Comparison. From the point of view of system integration current microfluidics is still far off from the level of early microelectronics and thus a general revolutionary potential can not really be expected. The poor system integration, on which the

most expensive sub-system, the control unit, is an off-chip part, the limited integration degrees and – as a particularly important reason – the absent decision-making ability do exclude that current fluidic ICs can carry out similar long, complex program sequences as early electronic microprocessors. Hence current microfluidics is mainly limited to applications like point-of-care diagnostics, which has benefits in usage such as portability, shorter time-to result, and less laboratory space consumption^{8,13,14} but no functional advantages.

We believe, that to push the LOC technology towards a level of a disruptive technology, new concepts and system architectures besides MEMS should be taken into consideration.

Fluidic microchemomechanical ICs. Here we present the concept of fluidic, microchemomechanical ICs. As in the case of MEMS LOCs, it shows similarities to the Von Neumann architecture. But, in addition – similar to early microelectronic computers based on microprocessors – it (a) integrates control unit and execution unit on one chip, (b) is suitable for large-scale integration and (c) employs the decision-making ability regarding chemical information (the carrier of information is a concentration) in fluidic ICs.

The system architecture consists of an execution unit, a control unit, an i/o unit and a power supply (Fig. 1C). Control unit, execution unit and chemical power supply are combined on a single integrated circuit acting as central processing unit. The IC processes two types of signals: chemical control signals and chemical data signals, which are – in our case – both part of the aqueous process media. The control signal is a binary chemical information, here the binary concentration of the water of the aqueous process medium ($c_{H_2O} = 0$: water is not applied; $c_{H_2O} = 1$: water is applied). The data signals are other parts of the process media, e.g. the concentrations of samples, chemicals, analytes,

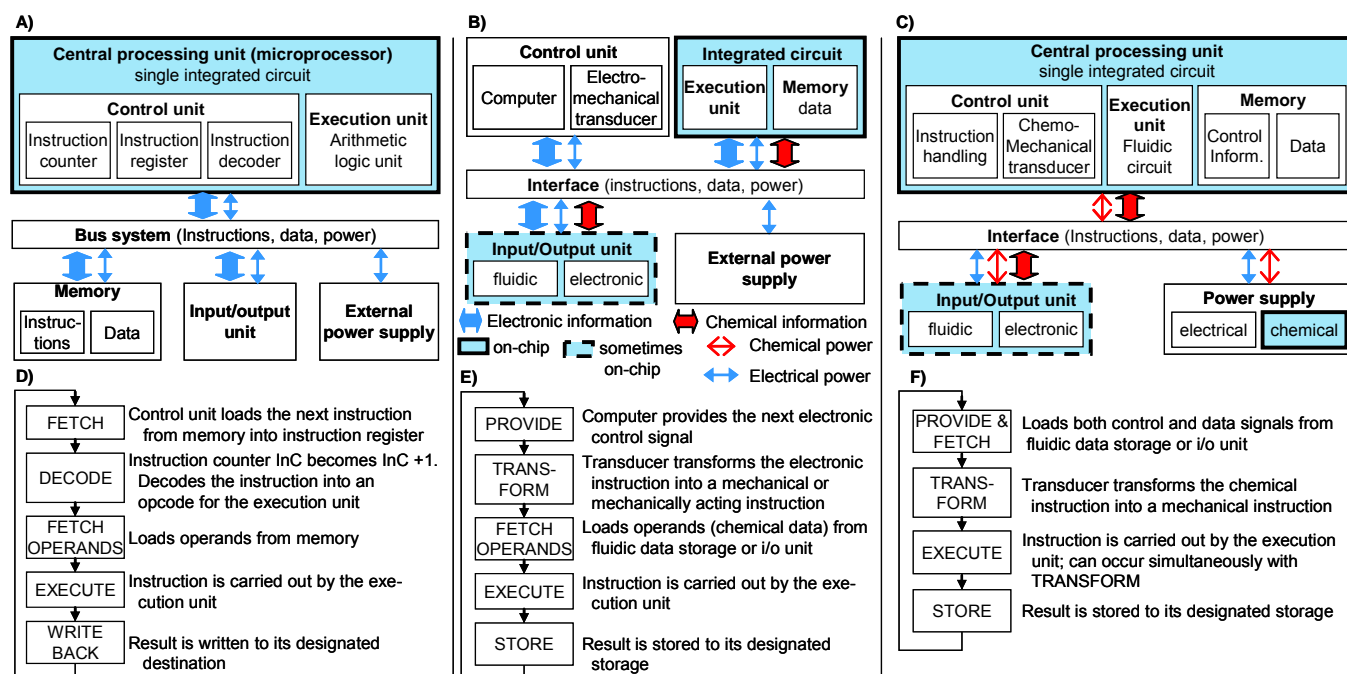


Fig.1 System architectures and basic cycles. (A–C) System architectures. (A) Electronic Von Neumann computer. (B) Microfluidic lab-on-a-chip system based on the concept of micro-electromechanical systems. (C) Microfluidic lab-on-a-chip system based on the concept of microchemo-mechanical systems. (D–F) Basic cycles. (D) Von Neumann cycle. (E) Basic cycle of a MEMS-based LOC. (F) Basic cycle of a μ CHMS-based IC. For MEMS and μ CHEMS the cycle need not be strictly sequential, since processes may occur continuously and overlapping.

which can be analogue carriers of information.

The basic cycle of a μ CHMS-based IC is as follows (Fig. 1F). Both control and data signal are loaded from the input or storage. The configuration and set-up of the active elements on the chip define an “instruction set”, i.e. they determine how an incoming control signal is transformed into transducer operation. The significant difference between MEMS and microchemomechanical LOCs is therefore the way how the instructions are activated (via electronic commands for MEMS, via a chemical control signal for μ CHMS). After execution of the mechanical instructions the processing of the chemical data can be run, e.g. as chemical or analytical reaction. Again, for abstraction, it is useful to group defined instruction sequences (e.g. those belonging to one module/cascade) as one complex instruction. In contrast to MEMS systems, these complex instructions are highly hardware-coded by the actuator and microfluidic circuit configuration.

This system architecture has two drastic consequences: both the entirely integrated control unit and the chemical power supply provide the processing of chemical information (a) completely energy self-sustaining and (b) self-controlled.

As in the case of microelectronics, the ability to realise microchemomechanical ICs with high complexity and functionality is based on intrinsically active materials, which are organised in several overlapping layers of different materials defined by microstructuring. Instead of active semiconductor materials with tailored electronic properties, e.g. doped silicon, which are the foundation of microelectronics, we use intrinsically active materials, the phase-changeable polymers,[‡] with specific sensor-actuator properties as the base for the active mechanically movable components. All components and circuits are built from a specific combination of these layers. This layer-by-layer fabrication allows to realise very complex monolithic ICs with thousands of components by minimum fabrication expenses.

Here we describe fabrication technologies, special properties and functionalities of the basic fluidic elements as well as basic modules of the microchip. As possible applications, we present two highly integrated microfluidic processors able to execute even complex processes with long program sequences. The first processor contains 2,096 microvalves organized in 192 serial connected cascades and is designed to perform long-term monitoring of critical process parameters over days or weeks by periodically sample analyses completely energy self-sustaining and self-controlled, whereas the second one has a mixed serial-parallel circuit structure and is able to monitor kinetic parameters over long times.

The microfluidic chips presented here are the first prototypes that exemplify the processing of chemical information by highly integrated μ CHMS. They give a proof of concept of integrated microfluidic processors that contain the control unit on-chip.

Materials and Methods

Materials

Sodium acrylate (97 %), *N*-isopropylacrylamide (NIPAAm) (99 %), crosslinking agent *N,N*-methylenebis(acrylamide) (99 %), photoinitiator 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (98 %), poly(ethylene glycol) PEG 6,000, 10,000,

20,000, 35,000, poly(vinyl alcohol) (87 -89 %, Mw 85,000 - 146,000), hydroxypropylcellulose, poly(acrylic acid), and pure ethanol (99.9%) were obtained from Aldrich. Distilled water was used to prepare photopolymerisable solutions. Fluorescein Standard, malonate and laccase were received from Fluka. Poly(dimethylsiloxane) was received as SYLGARD 184 Kit from Dow Corning. 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS, 98 %) was obtained from Aldrich. Poly(ethylene terephthalate) (PET) used for photomasks was obtained from Modulor.

Fabrication of the microfluidic chips

The monolithic microchips consist entirely of polymers. The substrates containing the channel networks are fabricated of poly(dimethylsiloxane) by means of large-area multi-layer soft lithography.^{19,21} The active components are composed of three different phase-changeable polymers that are integrated successively into the chip by means of layer structuring. Poly(ethylene glycol)s (PEG) are patterned by stencil printing, sodium acrylate (SA) gels are photo-lithographically patterned, and membranes consisting of poly(vinyl alcohol) (PVA) are incorporated employing transfer by using a slightly adhesive foil or pick-and-place.

Photopolymerisation of sodium acrylate hydrogels

The structuring of the sodium acrylate hydrogels was realised by photolithographic polymerisation. 2 g of sodium acrylate, 0.04 g of the cross-linking agent (*N,N*-methylenebisacrylamide) BIS, and 0.04 g of the photoinitiator (2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone) were dissolved in 14 ml distilled water. In the subsequent sections, this composition of the solution is referred to as c_0 . The solution was thoroughly stirred for 24 h and then purged with argon for 30 min to remove the free oxygen radicals. To perform the photopolymerisation, a polymerisation chamber ($h = 140 \mu\text{m}$, $w = 60 \text{ mm}$, $l = 60 \text{ mm}$) made of poly(ethylene terephthalate) (PET) was filled with the aqueous solution. The chamber was then closed by a 1 mm thick, transparent PET lid, covered with a structured photomask (TypoPhot TO-G, plotted by a Herkules Imagesetter with 5080 dots inch^{-1}), and placed under a Hg lamp HBO 100W/2 (Osram) equipped with a long-pass filter ($>200 \text{ nm}$), and an aperture to parallelise and bundle the UV light. After the polymerisation, the non-polymerised residues were removed by rinsing the obtained gels with a mixture of ethanol and water. The structures were dried for 24 h at 40°C in order to remove the moisture and solidify the hydrogels.

The photolithographic polymerisation of poly(*N*-isopropylacrylamide) has been described previously.²¹

The quality of the hydrogel layer depends on the polymerisation time, the distance to the light source, and on the thickness of the hydrogel layer. Optimal results have been obtained with an exposure time of 7 min at a 31 cm distance from the light source.

Stencil printing of poly(ethylene glycol)s

For the opening valves 3, 4 (Figs. 3, 6) melttable poly(ethylene glycol) is used as material base. To integrate the opening valves into the channel system, the method of stencil printing is applied. Here, a structured copper mask with a thickness of $20 \mu\text{m}$ is

placed onto the PDMS layer containing the channel systems. Then PEG which is heated above its melting temperature is cast onto the copper mask and is spread with a metal slide. Only the etched areas, which are dimensioned according to the desired size of the opening valves, allow a flow of PEG into the channel system; only at these defined spots opening valves are formed. As soon as the PEG touches the channel system, it cools down and hardens. The thereby integrated opening valves have the desired dimensions, but they do not seal the channel. To achieve hermetically sealed opening valves, the microprocessor has to be heated above the melting point of the PEG as a last production step. The PEG melts and seals the channel air tight.

Fabrication of membranes based on poly(vinyl alcohol)

To fabricate water-soluble membranes based on poly(vinyl alcohol), a 5 % polymeric solution is cast onto a horizontally aligned glass substrate equipped with 5 mm high silicon walls and is left there to dry. After 3 days, the water of the solution has evaporated and has left a thin PVA membrane whose height can be adjusted by the amount of the solution cast onto the glass substrate. To fit the microprocessor, the membranes are structured with a wafer saw and can then be transferred to the microprocessor by a slightly adhesive foil or even - for lab reasons only - manually incorporated.

Design of the microprocessor for equidistant long-term investigations

The microchip shown in Fig. 5 has a two-layer architecture. The upper layer 1 supports the channel structure of the circuit for medium 1, while the lower layer 2 contains the circuitry of medium 2. Both layers have a similar design. The channels are 800 μm wide and 140 μm high. The bypass channels are 400 μm wide and 140 μm high. The quadratic rhombuses of the closing valve chambers have a volume of 1,000 x 1,000 x 140 μm^3 (valve 1) and 800 x 800 x 140 μm^3 (valve 5). The configuration of the active components used for the experiment shown in Fig. 5 is as follows: the thickness of the membrane 2 based on poly(vinyl alcohol) is 70 μm (dissolution time 7 min). The length of the opening valves 3 made from PEG 6,000 is 400 μm (the intended opening time was 2 min at a flow rate of 30 μLmin^{-1}), the length of the valves 4 based on PEG 6,000 is 800 μm . The dry sodium acrylate actuator of valve 1 has a volume of 500 x 500 x 100 μm^3 (volume ratio 1 : 5.6, closing time 45 s), the actuators of valves 5 are 240 x 240 x 100 μm^3 in size (volume ratio 1 : 16, closing time 3 min).

Design and operation of the microprocessor for kinetic investigations

The microchip consists of two layers (Fig. 7B). The lower layer 1 supports the channel structure, while the upper layer 2 contains the storages of the immobilised substrate.

The main channels are 800 μm wide and 140 μm high. The channels leading to the reaction chambers are 400 μm wide and 140 μm high. The quadratic rhombuses of the chambers of valve 1 have a volume of 700 x 700 x 140 μm^3 . The dry sodium acrylate actuator of valve 1 has a volume of 300 x 300 x 100 μm^3 (volume ratio 1 : 7.6, closing time 1 min). The length of the opening valve 4 made from PEG 6,000 is 600 μm (opening time 5 min at a flow rate of 30 μLmin^{-1}).

Preparation of the storages of substrate (microprocessor 2)

The substrate 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (2 mM in 0.05 M malonate buffer) is pipetted into the storage wells (reservoirs) of layer 2 (from left to right: 15 μL , 10 μL , 5 μL , 5 μL , and 5 μL) (Fig. 7B). After drying the solution (10 min at room temperature) layer 2 is ready to be connected to layer 1.

Protocol of the laccase test (microprocessor 2)

The sample solution consisting of the enzyme laccase (dissolved in 0.05 M malonate buffer) and fluorescein is inserted into the fully assembled chip. This solution passes and activates the opening valve (Fig. 7B), which defines the period between two sampling steps, and flows into the splitting chamber. Here, the flow is divided into six channels. Five of them lead to the reaction chambers, one is used as a bypass. This bypass allows a continuous flow even after the reaction chambers are closed and thus ensures the timed dissolution of the opening valve. In the five channels leading to the reaction chambers, the flow passes and activates the closing valves. After filling the analyte chambers, which contain the various amounts of the dried substrate, the flow reaches and activates the second row of closing valves. After the closing valves 1 have sealed the reaction chambers, the dried ABTS dissolves in the sample solution and activates the enzyme laccase.

Results

Active components

For the closing valves we utilise the principle of chemomechanical valves, which have already been proposed in the beginning of the eighties²² and fully demonstrated in 2000.^{23,24} These valves are based on very special materials, the stimuli-responsive or smart hydrogels, which exhibit volume phase transition behaviour. Even a small change of certain environmental parameters, e.g. a concentration of a solvent or the pH value, leads to a drastic, reversible and reproducible change of the volume of the smart hydrogel. This swelling or shrinking of the hydrogel component allows to regulate a continuous liquid flow by variation of a channel cross-section.²⁵

Here, we generalise the principle of chemomechanical components by significant expansion (a) of the material base to the much larger material class of phase-changeable polymers and (b) of the functionalities of the components. Our active components feature multiple functionalities: they are state-switchable elements, that, by transformation, release or consume energy, a timed and quantitative exactly defined fluidic function can thus be realised. The activation of each fluidic component - and also the control of the complete fluidic circuitry - results from chemical signals. Hence the component has a combined sensor-actuator functionality and is suitable to realise a discrete, binary coding paradigm.

Closing valves

Our microchips consist of different types of microvalves (opening or closing valves), the type of each valve is defined by the chosen phase-changeable polymer. When activated, closing valves (Fig. 2A) are intended to close a microfluidic channel. They are based

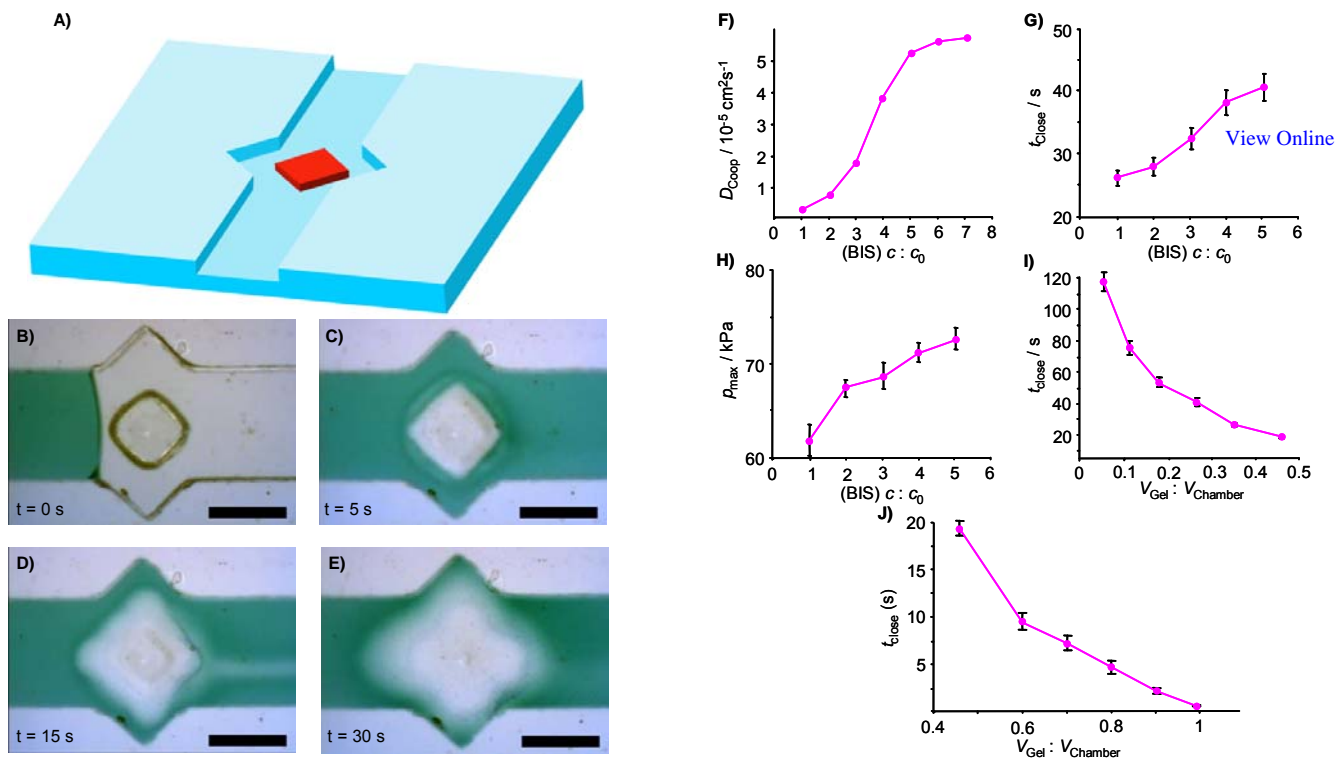


Fig. 2 Characteristics of closing valves based on photo-patterned hydrogel sodium acrylate (SA). (A) Schematic of the valve. (B-E) Closing process, scale bars 500 μm . (B) Initial state of the closing valve immediately before its activation by switching the binary concentration from 0 (no water) to 1 (water is applied). (E) Closed valve. Due to the presence of aqueous solution, the hydrogel actuator is completely swollen and blocks the liquid flow (F) Cooperative diffusion coefficient D_{Coop} of a SA hydrogel depending on the reference concentration $c : c_0$ of the cross-linking agent BIS, with $c_0 = 2$ g of SA, 0.04 g BIS, and 0.04 g of the photoinitiator (2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone) dissolved in 14 ml distilled water. (G) Closing time t_{close} and (H) back pressure p_{max} of SA-based valves (exposure time 7 min, hydrogel volume $600 \times 600 \times 100 \mu\text{m}^3$ in dry state, volume ratio hydrogel : chamber 0.25) depending on the reference concentration $c : c_0$ of the cross-linking agent BIS. (I-J) Closing time t_{close} as a function of the ratio of the hydrogel volume (BIS 1) in its dry state to the volume of the valve chamber $V_{Gel} : V_{Chamber}$.

on cross-linked, water-swallowable polymers, so-called hydrogels, in which single polymer chains are linked to form three-dimensional networks. Upon contact with a fluid, the hitherto dominating polymer-polymer interactions between the chains of the polymer network are being replaced by polymer-solvent interactions. This energy conversion results in a significant increase of expansive energy contributions inside the gel,²⁶ it swells and performs mechanical work by closing the valve (Figs. 2B-E, see video S1)†.

Its closing time can be basically predefined by the hydrophilicity and cooperative diffusion coefficient of the chosen polymer. Two types of hydrogels can be distinguished, neutral and polyelectrolyte hydrogels. Neutral hydrogels such as poly(acryl amide), poly(*N*-isopropylacrylamide) (PNIPAAm), poly(methyl vinyl ether), poly(vinyl alcohol), or poly(ethylene glycol) have cooperative diffusion coefficients (D_{coop}) that are typically in the order of $10^{-7} \text{ cm}^2 \text{ s}^{-1}$.^{27,28} Polyelectrolyte hydrogels, e.g. sodium acrylate (SA), comprise ionisable groups, e.g. weak acidic or basic groups. The contributions of the electrostatic interactions within the polyelectrolyte gels lead to cooperative diffusion coefficients in the range of 10^{-7} to $10^{-5} \text{ cm}^2 \text{ s}^{-1}$.

According to Tanaka the time constant of the swelling process of hydrogels

$$\tau \sim \frac{d^2}{D_{Coop}} \quad (1)$$

depends on the square of the characteristic dimension d of the gel-based component, which is in the case of a spherical particle

the radius.²⁸ In the interesting range of d of few hundred microns, neutral hydrogels are a suitable material base for relatively slow valves with closing times in the range of minutes or hours, whereas polyelectrolyte hydrogels are predestinated for fast valves with closing times in the millisecond to minute range. It is remarkable, that the D_{Coop} of the polymer networks are in the same order of magnitude as the self-diffusion coefficients D_{Self} of molecules important in microfluidics $\{D_{Self}(\text{Na}) = 1.3 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $D_{Self}(\text{H}_2\text{O}) = 2.27 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $D_{Self}(\text{Bovine serum albumin}) = 6.37 \cdot 10^{-7} \text{ cm}^2 \text{ s}^{-1}$, $D_{Self}(\text{Deoxyribonucleic acid}) = (10^{-8} \dots 10^{-11}) \text{ cm}^2 \text{ s}^{-1}\}$.²⁹⁻³² This ensures that, independent of the length-scale of the microfluidic processor, the active components can operate on the same time-scale as the processes driven by self-diffusion.

D_{Coop} of the sodium acrylate hydrogels depends on the cross-linking conditions, especially on the concentration of the cross-linking agent *N,N'*-methylenebisacrylamide (BIS) (Fig. 2F). The more cross-linking agent BIS is used, the higher is the cooperative diffusion coefficient and the smaller the time constant τ . At a reference concentration $c : c_0 = 7$ of the cross-linking agent BIS, additional cross-linking agent does not influence the cooperative diffusion coefficient any more. With a cooperative diffusion coefficient of 0.2×10^{-5} to $5.7 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (calculated by the theory of Tanaka²⁸), the hydrogel shows a very fast swelling behaviour compared to other swellable hydrogels used in microfluidic devices.²⁶

The closing time of a valve depends both on the time constant τ and the maximum swelling degree that the hydrogel has in free swelling. A higher cross-linking degree of the polymer leads to a

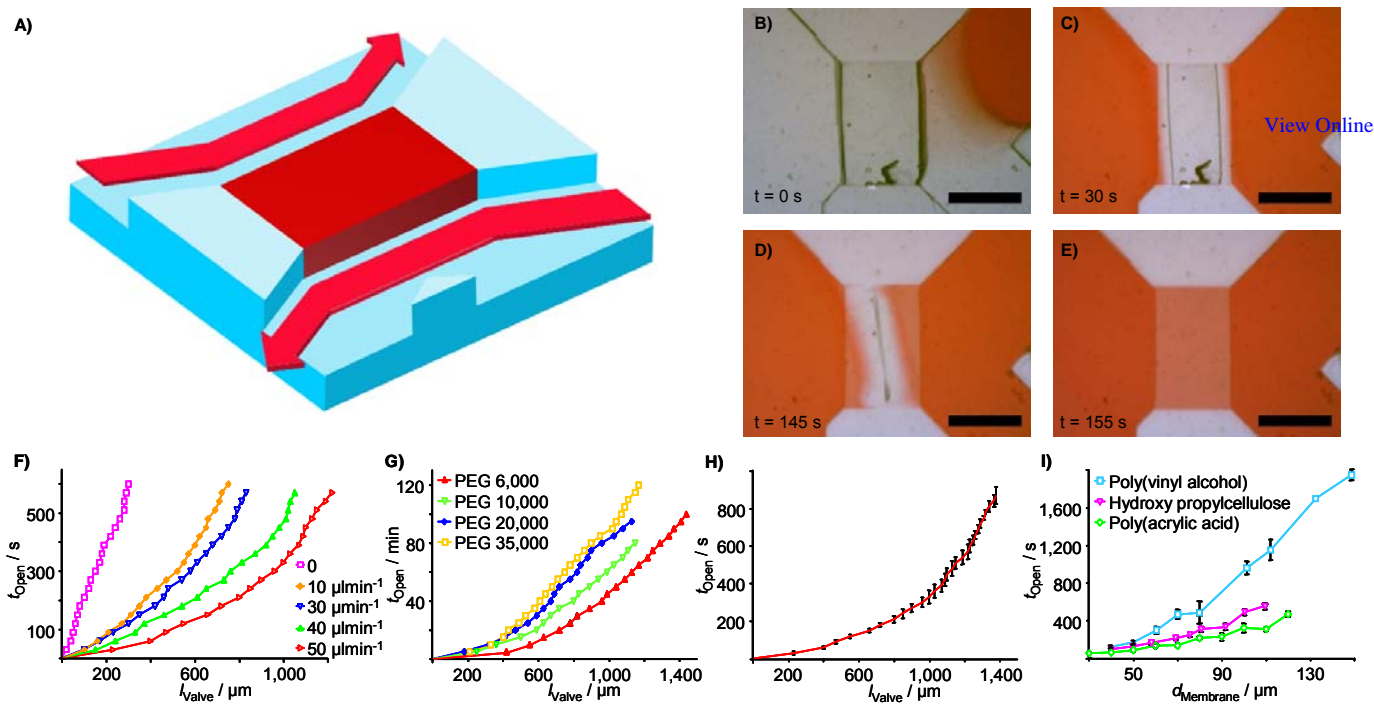


Fig. 3 Characteristics of opening valves based on dissolvable polymers. (A) Schematic of the valve. (B–E) Opening process, scale bars 500 μm . (F) Opening time t_{open} depending on the valve length l_{valve} and the flow rate of the process medium (valves based on PEG 10,000). (G) Opening time t_{open} of valves based on different water-soluble PEG polymers as a function of the valve length l_{valve} . (H) Standard deviation of the opening time of a valve made from PEG 10,000 for different valve lengths at a flow rate of 50 μlmin^{-1} . (I) Opening time t_{open} of membrane valves depending on the used water-soluble polymer and the membrane thickness d_{membrane} .

stiffer hydrogel and therefore reduces the maximum swelling degree. For the dependence of the valve closing time t_{close} on the cross-linking agent concentration (Fig. 2G) the decrease of the maximum swelling degree with higher $c : c_0$ is the dominating effect compared to the increase of the diffusion coefficient.

Fig. 2H illustrates that the higher the stiffness of the hydrogel actuator, the higher the back pressure of the closing valve (maximum pressure that can be applied without any leakage flow of the valve).

The precise adjustment of the closing time of a hydrogel-based valve is achieved by a special design parameter, the volume ratio of the polymer in its dry initial state compared to the valve chamber. As shown in Figs. 2I–J for the polyelectrolyte hydrogel sodium acrylate the adjustment of the closing time of the valves is very versatile in a wide range spanning from minutes to milliseconds. Opposite that, by utilising neutral hydrogels, e.g. PNIPAAm, with smaller D_{coop} than polyelectrolyte hydrogels, relatively slow valves with closing times in the range of minutes to hours should be realisable.

As we will see in the description of the ICs, for the design of LOCs based on μCHMS , it is significant to have elements whose response time can be scaled over a big range.

Opening valves

Opening valves (Fig. 3A) consist of dissolvable polymers. Because their molecules are not cross-linked, the breakup of their intermolecular polymer-polymer interactions leads to the dissolution of the polymer and thus to an opening of the valves (Figs. 3B–E, video S2)†. The opening time of opening valves can be defined by valve length and depends also on the flow rate of

the passing process medium (Fig. 3F).

In a stagnant process medium (flow rate = 0) at the interface of the polymer valve, a saturation zone develops, which impedes the dissolution of the polymer. With increasing flow rates this saturation zone decreases or disappears and the dissolution or opening time of the valve decreases. Additionally, with increasing flow rate the elution of polymer chains increases resulting in faster opening times. In contrast to the opening valves, the time behaviour of closing valves is not influenced by the flow rate of the process medium.

Moreover, the general time behaviour of the valves is predefined by the chosen soluble polymer. The opening time of a valve increases with increasing chain length of PEG and thus increasing hydrophobicity of this polymer (Fig. 3G). Due to their wax-like consistency, PEG-based valves of our microprocessors are integrated by stencil printing. For the second type of opening valves a thin, stiff and water-soluble membrane is needed. PEG is too soft a material and not consistent enough in form to serve as a material for a membrane. Ideal candidates are polymers with a glass transition temperature considerably higher than room temperature. Poly(vinyl alcohol) ($T_g = 85^\circ\text{C}$), hydroxypropylcellulose ($T_g = 105^\circ\text{C}$), and poly(acrylic acid) ($T_g = 105^\circ\text{C}$), for example, are suitable materials.³³ Besides, these polymers provide the possibility to fabricate membranes with a defined thickness by simply casting and drying the polymeric solutions. The dissolution/opening time of membrane valves based on these materials depending on the membrane thickness is shown in Fig. 3I. This time is predefinable in the range from a few seconds to several hours.

Energy density of intrinsically active polymers inside valves

An important parameter of an actuator is its energy density. In principle, the higher the energy density, the more versatile and the better scalable is the actuator.^{34,35} The effective energy density of actuator components inside valves can be estimated by

$$e_d = \frac{dE}{dV} = \frac{p \cdot A_v \cdot l_0 \cdot (1 - a_{vol})}{V_v} \quad (2)$$

with p - maximum back pressure of the valve; A_v - pressurised valve area; l_0 - valve length; V_v - valve volume; a_{vol} - volume ratio, which is for (a) closing valves (swelling actuators): ratio of dry volume of the actuator material in the initial state to chamber volume, (b) opening valves (dissolvable polymers): $a_{vol} = 0$, since the polymer actuator will be dissolved completely (change of volume equals 100 %). The energy densities give an indication of the pressure resistance, that has to be overcome to completely open a valve mechanically.

The maximum back pressure and the resulting effective energy density of a swelling intrinsically active polymer inside a closing valve are shown in Figs. 4A, B on the example of PNIPAAm.

The energy density of the active polymer depends not only on its actuator properties, but also on design parameters, here the volume ratio a_{vol} and the material used as body material. For the experiments shown in Figs. 4A, B Si is used as valve body material. This material is rigid and allows to perform microfluidic investigations at high pressure. Valves placed inside a PDMS substrate as used for our microchips provide maximum back

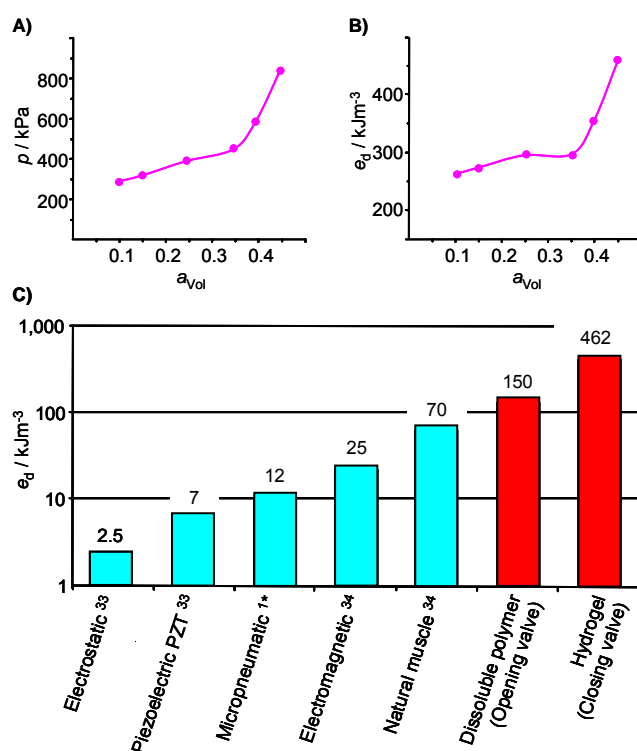


Fig. 4 Effective energy densities of the used intrinsically active polymers. (A) Back pressure p of a closing valve based on the hydrogel poly(*N*-isopropylacrylamide) (valve body material: Si). (B) Resulting effective energy density of the PNIPAAm-based closing valve. (C) Comparison of the energy densities of actuators often used in LOC technology. * calculated from¹

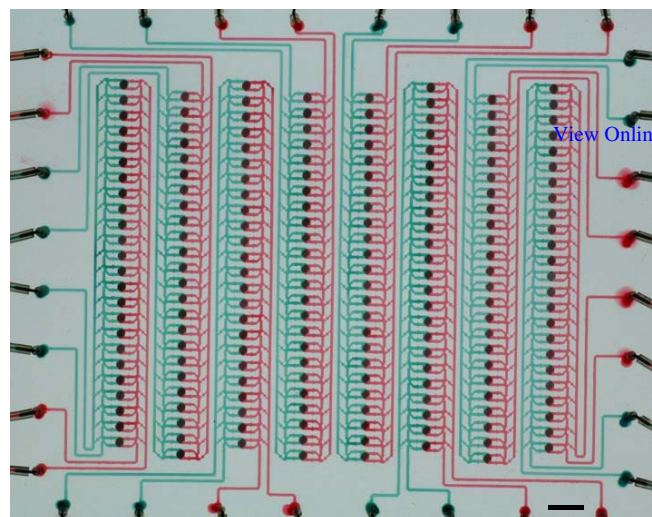


Fig. 5 Photograph of the fluidic microprocessor for equidistant long-term investigations. The different channels are filled with food colouring. Scale bar 10 mm.

pressures of about 150 to 200 kPa. This limitation of the back pressure results from the softness of PDMS, which leads to an unwanted opening of the valve due to the elastic substrate deflection.

The effective energy densities of dissolvable polymers inside opening valves are in the same order of magnitude as those of the hydrogels. In our microchips, the maximum back pressure of valves based on PEG 6,000 is about 150 kPa, this corresponds to an effective $e_d = 150$ kJm⁻³.

It is remarkable, that intrinsically active polymers provide energy densities, which are larger than that of the current actuator principles used in LOC including piezo, micropneumatic, electromagnetic, electrostatic actuators or even the human muscle (Fig. 4C). Additionally, in contrast to MEMS-based approaches, no extra volume for the electronic/electromechanical/pneumatic actuation units is necessary.

Microchip for equidistant long-term investigations

The microchip shown in Fig. 5 is designed to carry out long-term monitoring of critical parameters by around-the-clock sampling and analysis in completely self-controlled and energy self-sustaining manner. Equidistant sampling and analysis belong to the most common tests in science to control critical variable parameters, e.g., monitoring of bioreactors, enzyme analysis, analysis of factors of growth, or quality control of chemical and biological products. However, these long-term, around-the-clock investigations are usually performed by lab staff, and are therefore time-consuming and labour intensive.

The microchip consists of 192 serial connected cascades each able to carry out sampling exactly once, with a total of 2,096 microvalves and 384 reaction chambers of 882 nl each (Fig. 5). For lack of space the cascades are organised in 8 columns a 24 cascades, which can be connected serially with each other or with up to 8 different bioreactors. The microchip has a monolithic structure and consists entirely of polymers. The substrates containing the channel networks are fabricated of

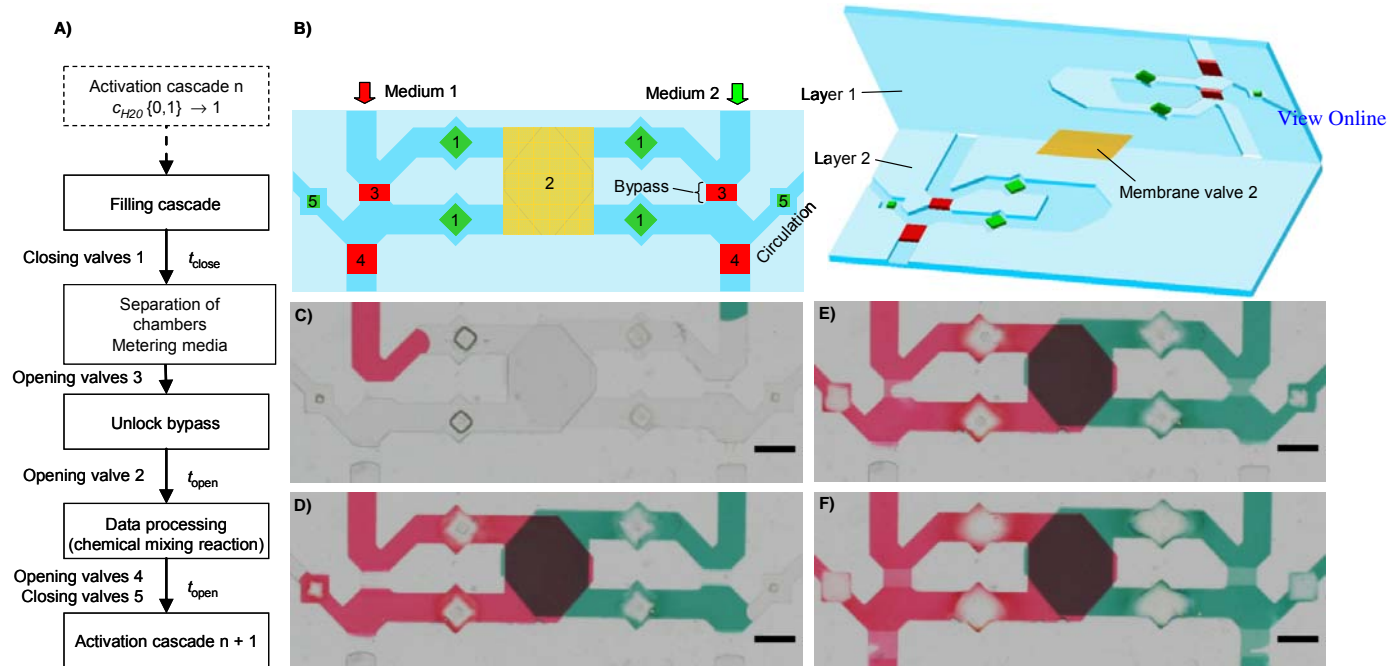


Fig. 6 Functional principle of one cascade of the microprocessor. **(A)** Cycle of a cascade. The complex instruction "sample and analyse" consists of five elementary instructions. **(B)** Schematic of the cascade, left - top view, right - 3D view. **(C)** The process media, which contain both the binary control signal and the data signals, *e.g.* sample and analyte, reach the cascade. The control signal, the binary concentration of water c_{H_2O} , switches from 0 to 1 and activates the cycle of the cascade. After that, the process medium floods the reaction chambers. **(D)** After a defined time delay, in this case after about 45 s, the valves 1 close, separate the completely flooded chambers and meter the process media as well as the chemical data. **(E)** The increase of pressure after closing valves 1 leads to a rapid breakthrough of opening valves 3, which quickly dissolve and thus open the channels that bypass the chambers. As soon as the membrane valve 2 that separates two reaction chambers has dissolved, the data containing process media intermix and the data processing in form of a chemical mixing reaction starts. **(F)** The opening valves 4 dissolve after a predefined time delay. This determines the period before the next cascade is activated. At this moment, the slowly working valves 5 close the stage's bypass channels necessary for the circulation of the process media. Scale bars 1 mm.

poly(dimethylsiloxane) by means of multi-layer soft lithography,^{19,21} as active components the closing and opening valves described above are integrated into the chip.

Each cascade consists of two functional groups, (a) the operation group and (b) the timing group.

The operation group, which comprises the valves 1 - 3 and the reaction chambers (Fig. 6B), realises all fluidic operations that are necessary for the sampling, the sample preparation and the conduction of the reaction, and defines the sequence of the operations. The time-defining function of the operation group leads to requirements on the temporal behaviour of its active components, which are very different from those of MEMS-based LOC. Looking to MEMS-based LOC, the time control of the active components such as valves is carried out by the control program of the computer, hence valves with a fast response of the instructions are advantageous. For our IC concept we do not need quick active components. It is rather necessary to have versatile components, whose time behaviour can be exactly tailored in a wide range of time.

The basic "sample and analyse" cycle and its elementary instructions are explained in Figs. 6A, C-F and shown in the video S4.† The basic cycle starts, when the binary control signal, the binary concentration of water c_{H_2O} , switches from 0 to 1 respectively. The closing time of valves 1, based on SA, is tailored exactly to the time required to completely fill the reaction chambers and to pass the valves 3 and 4 to avoid malfunction. We dimensioned the closing time of valves 1 of 45 s ($a_{Vol} = 1 : 5.6$) for a minimum flow of the process medium of $1.5 \mu\text{L min}^{-1}$, which completely fills both the reaction chamber and

the channel part to valves 3, 4 in 45 s.

Membrane valve 2, separating two adjoining chambers, is based on PVA. Its dissolution time of 7 min (membrane thickness $70 \mu\text{m}$) compensates for potential time differences during the filling of two adjoining chambers. We used only one multi-channel hose pump (ISMATEC IPC ISM 936D) to guaranty that the flows of both the red and green process media are identical.

The valves 3 have a special function. In order to fill the reaction chambers, opening valves 3 placed inside the chamber-bypasses are initially closed. As soon as valves 1 have closed the chambers, the bypasses have to be unlocked so that the fluidic circulation is not interrupted. The opening of these valves 3 is controlled by the increasing pressure in the bypasses leading to a breakthrough of the valves which then rapidly dissolve (Fig. 6E, video S3)†. Valves 3 are essential components for sequential circuits with many cascades. Without them, the flow would have to be guided into the reaction chamber by choosing a high resistance for the chamber-bypass. In consequence, the rapidly growing resistances of the fluidic channels would only allow for microfluidic processors with a maximum of three or four cascades.

The timing group consists of valves 4 and 5 and specifies the time interval between the cycles of two consecutive stages. When the valves 4 open, the next cascade is activated. The slowly working valves 5 close the stage's circulation lines. The closing time of valves 5 is tuned ($a_{Vol} = 1 : 16$) in accordance to the opening time of valves 4 (Fig. 6F). It is also possible to use valves 5 to predefine the time interval between two samplings by enforcing a breakthrough of valves 4 in the same manner as

described above.

In the current set-up the chip takes samples at intervals of 3 min. By exploiting the versatility of the valves the time behaviour of the chip can be scaled to operate for a maximum of 16 days, performing automatically analytical reactions at two-hour intervals (valve 4 PEG made of 35,000 and 1.2 mm in length).

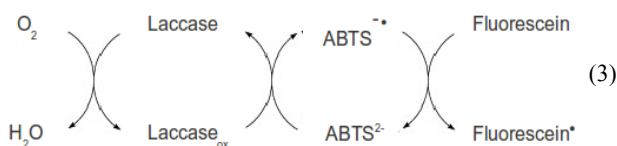
The sampling of the chemical input concentration at regular intervals corresponds directly to the sampling of a signal in electronic data processing. In a typical operation scheme of the chip channel 1 contains the sample with a varying chemical concentration, while the chemical in channel 2 will have constant concentration. The concentration of chemical 1 at the moment of sampling can then be determined by an endpoint analysis of the reaction of the two chemicals in the different chambers.

The microfluidic chip can be regarded as a microprocessor for chemical information according to Fig. 1, since control unit and execution unit are integrated on one chip.

Microchip for equidistant long-term kinetic investigations

The second microchip (Fig. 7A) is able to perform around-the-clock kinetic rate measurements by 64 sequential reaction complexes each consisting of five variable reaction conditions. In contrast to the first microchip here we do not mix two liquids, but one liquid and an immobilised component. The immobilised component of the reaction system is placed in the reservoirs of the five reaction chambers (Fig. 7B). The volume ratios of each reaction chamber to reservoir are different and thus each reaction chamber provides a different, but defined amount of the immobilised component. The liquid contains the reaction components like enzyme, substrate and buffers without one variable factor that is in the immobilised phase. As in the case of the first chip, one multi-channel hose pump of type ISMATEC IPC ISM 936D was used to pump the fluid.

We explain the functionality of the microchip on the example of optimization of a new enzyme test. We use the enzyme laccase, a monophenol oxidase of the fungus *Trametes versicolor*, which is able to catalyse the one electron oxidation of ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) (Fig. 7).^{36,37} The produced radical ABTS^{\bullet} bleaches fluorescein and is reduced to ABTS^{2-} :



Measured is the reduction of fluorescein fluorescence. To use this test the concentrations of each component of the reaction have to be optimised. ABTS functions as a mediator for single electron transfer. When it is used in too small quantities, it influences the velocity of the reaction. To find the needed amount of ABTS we tested different concentrations. Therefore different quantities of ABTS were immobilised in the reaction chambers. The test starts when the aqueous process medium with the residual reaction components flows into the chambers. The valves close, separate the reaction chambers and thus meter the liquid. Now the immobilised ABTS inside the reservoirs starts to dissolve leading

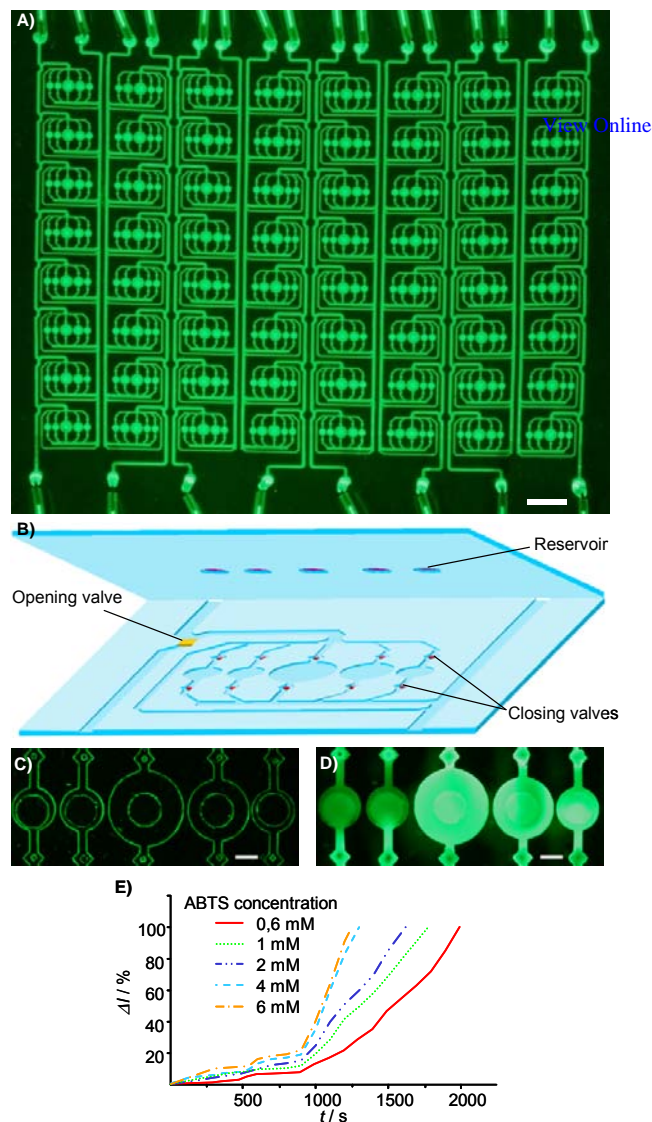


Fig. 7 Microchip designed for an equidistant analysis of the kinetics of reaction systems. (A) Photograph of the microchip filled with a fluorescein containing liquid. Scale bar 10 mm. (B) Schematic of one stage. (C) Photograph of an unfilled stage. (D) Photograph of a filled stage after the dissolution of the substrate. Scale bar 1 mm. (E) Time-dependent change of signal intensity ΔI of the five reaction chambers representing different volume ratios of sample containing solution and substrate (sample : substrate).

to final concentrations of 0.6; 1.0; 2.0; 4.0 and 6.0 mM in the five chambers. ABTS reacts with laccase and the produced ABTS radical bleaches the fluorescein, resulting in a decrease of the fluorescence signal. As shown in Fig. 7E the dissolution of the mediator occurs in 15 min. After this time, the reaction starts showing a linear increase of the reaction signal. Depending on the ABTS concentration different velocities of the reactions are observable. For the two highest concentration the reaction reaches nearly the same velocities. Therefore the concentration of ABTS needed to function properly as a mediator is $> 4 \text{ mM}$.

The presented microchip can also be used for the optimisation of different test parameters like laccases, mediators, fluorescent dyes, inhibitors of laccase, buffers and used pH or ionic strength. E.g. in each of the 8 cascades of one column we are able to analyse 8 different substances like fluorescein, rhodamine, eosin,

aesculin etc. as fluorescent dyes. Hence test optimisation on a wide array of parameters is possible with one chip.

With the accessible data, kinetic parameters can be comparatively analysed like maximal velocity v_{max} , Michaelis Menten constant K_m or inhibitor constants of the given reaction system by using the Lineweaver-Burk plot.³⁸⁻⁴⁰ For an absolute calculation of these parameters it is advisable to neglect the time interval of dissolution and evaluate the remaining data points by a fitting algorithm.

The function of the opening valve (see Fig. 7B) depends on the application of the chip. If a complex analysis with a large analyte set should be performed as fast as possible, the opening time should be dimensioned, such that it guarantees the complete filling of all chambers of the cascade. For around-the-clock analyses the opening time determines the sampling time.

The operation of this chip shows that in addition to serial processing also parallel processing in one cascade is possible.

Conclusions

We introduce the microfluidic single-chip system architecture of a central processing unit, which contains both control and execution unit and moreover the complete chemical power supply of the chip. We hope that the concept of a central processing unit leads to a drastic increase in the performance of LOC technology, as it happened in microelectronics. The conditions are good. The layer-by-layer fabrication of monolithic microchemomechanical ICs based on several overlapping layers of different intrinsically active polymers with tailored sensor-actuator properties is excellently scalable and is therefore an appropriate technology for microchips with increasing complexity. Additionally, the high energy density of our intrinsically active polymers, which exceeds that of actuator systems hitherto applied in LOC-technology significantly, facilitates a good scale down of components and their versatile usability. Compared to early microelectronics, the integration degree of the microchip shown in Fig. 4 consisting of 2,096 valves is almost identical with that of the first commercial electronic microprocessor, the Intel 4004, containing 2,300 transistors.¹⁰

As a second significant novelty the concept of microchemomechanical ICs introduces a new paradigm of information processing into microfluidic ICs: the active processing of chemical information based on the decision making ability of the basic components. This principle is very powerful. The microprocessor shown in Fig. 5 can carry out sequentially repeated 192 samplings in completely self-sufficient and automatic manner. From the point of view of circuit design this task is very challenging, since controlling 192 sequential cascades with eleven components each needs a large address space. A micropneumatic, multiplexed chip controls n fluid channels with

$$N = 2 \log_2 n \quad (4)$$

control channels.¹ For the independent control of 2,096 valves $N = 24$ pneumatic control channels are necessary, which requires complex and expensive machinery (pneumatic control device, computer and control software).

The foundation stone of ICs processing chemical data lies in

the active components with decision making ability. Future research will profit from applying the concepts of existing hydrogel components, such as chemostats⁴¹ and chemical transistors^{25,42} to integrated microfluidic chips. The option of controlling the chemical threshold value thermally or electronically looks especially promising. [View Online](#)

From a general point of view, our microchemomechanical ICs extend the possibilities of information technology by a novel "Beyond CMOS"⁴³ hardware approach of central processing units, which process non-electronic carriers of information. The processing of chemicals as carriers of information is a very interesting computing paradigm, since it is the basic principle of the information processing of living organisms, where it is characterised by technically unrivalled power, resilience and reliability.⁴⁴ Furthermore, many problems of today's natural and life science, which are based on chemical information processing, are too complex to solve with simulations performed by electronic computers. In our vision chemical microprocessors will be helpful to solve these and many other problems.

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† Electronic Supplementary Information (ESI) available:

Video S1: Operation of a closing valve 1 (Figs. 2, 6) with a sodium acrylate actuator in real time. The quadratic rhombus of the closing valve chamber has a volume of $1000 \times 1000 \times 140 \mu\text{m}^3$ (width x length x height).

Video S2: Operation (real time) of an opening valve 3 (Figs. 3, 6) with a PEG 6,000 actuator. The actuator has a volume of $800 \times 300 \times 140 \mu\text{m}^3$ (width x length x height).

Video S3: Pressure driven opening of a valve 4 (Figs. 3, 6) (real time).

Video S4: Operation of one stage of the microprocessor shown in Figs. 5-6. This video is a compilation of a series of single photos and thus a time lapse video. See DOI: 10.1039/b000000x/

‡ Polymers, which are able to change their material properties, e.g. volume or mechanical properties, at least once, without the necessity of phase transition behaviour and substantial/shape coherence.

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