Molecular Devices

DOI: 10.1002/anie.200703813

Fluorescent Molecular Logic Gates Using Microfluidic Devices**

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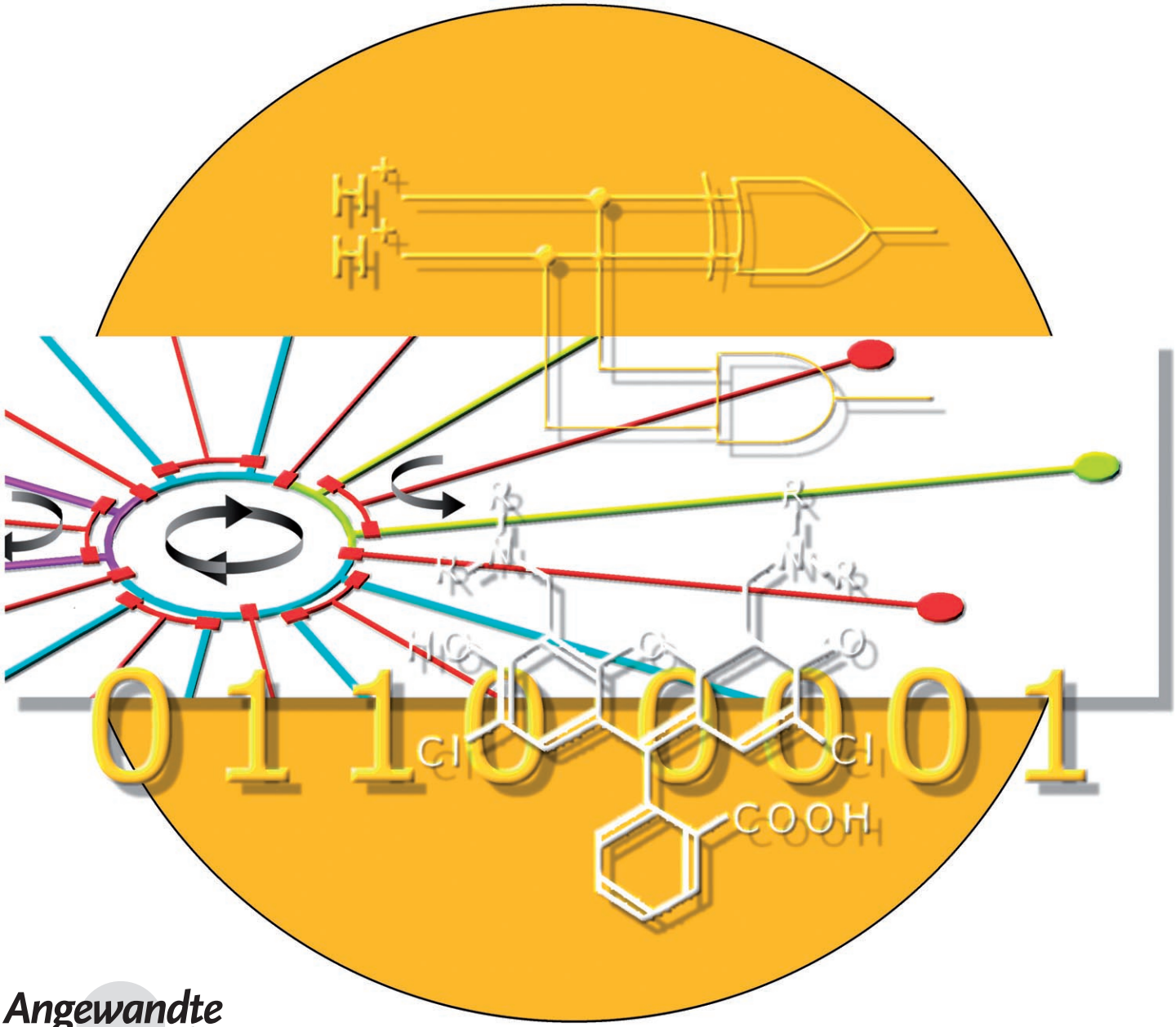


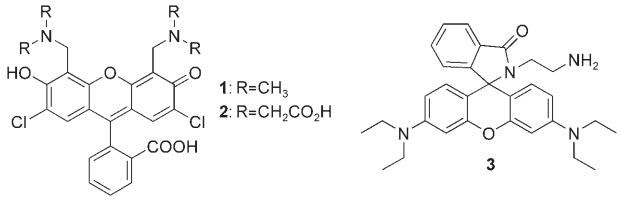
Since the pioneering work by de Silva et al.,[1] remarkable progress has been achieved in the development of molecular logic gates. Chemists have reported that a molecular logic gate has the potential for calculation on the nanometer scale, which is unparalleled in silicon-based devices. Various molecular logic gates (AND, OR, XOR, NAND, NOR, INHIBIT, half adder, half subtractor, etc.) that employ fluorescence changes have been studied intensively using various inputs, such as pH, metal ions, and anions.[2,3] The previous results of fluorescent chemosensors for ions have provided important tools for the development of molecular logic gates.[2]

On the other hand, microfluidic systems are increasingly used in the fields of analytical chemistry and biomedical sciences. These miniaturized chemical analysis systems (lab-on-a-chip) are already replacing complex, bulk equipment[4] and can provide simple point-of-care devices.[5] A microfluidic system consists of a network of microchannels. On account of its micrometer-scale size and therefore the small quantities of reaction solutions required (in the picoliter range), a microfluidic system provides an excellent platform, for example, for high-throughput screening[6] and the study of reaction kinetics.[7] The addition of microvalves and pumps[8] to the system enables precise process control, which directs the solutions in the reaction chambers and mixers. Such a system is capable of performing relatively complex processes, such as biological analysis,[9] synthesis,[10] and detection.[11]

With programmable microfluidic systems, an analogy can be drawn with electronic computers: the microfluidic channels are the wires that distribute the information (reaction solutions), while the reaction chambers or mixers are the logic operators.[12] Logic gates have also been constructed with redox compounds.[13] Other studies on logic operations include the use of two-phase flows containing droplets,[14] bubble logic,[15] and biomolecular computing.[12c]

In the study presented herein, a molecular logic gate in a microfluidic system was constructed based on fluorescent chemosensors by detecting the changes in intensity as a response to various inputs (pH, metal ions). This system was implemented in a programmable microfluidic device.

An XOR logic gate was demonstrated in a microfluidic system by controlling the pH of a solution of a fluorescein derivative **1** (Scheme 1), which was then advanced to a combinatorial circuit, such as a half adder using fluorescein derivative **1** and a rhodamine B derivative **3**. An INHIBIT logic gate was also demonstrated with OH^- and Cu^{2+} ions as inputs. Finally, the first example of a molecular logic gate using a protein and Cu^{2+} ions as inputs was demonstrated, in which a Cu^{2+}-selective fluorescein derivative **2** (Scheme 1) was employed as the communicating signal material. The fluorescein derivatives **1** and **2**[16] were synthesized by reported procedures. Rhodamine ethylamine **3** was also synthesized according to a reported procedure.[17]



Scheme 1. Structures of compounds **1**–**3**.

A microfluidic device to mix two or more different fluids (Figure 1a) consisted of two layers of polydimethylsiloxane (PDMS), and was fabricated using multilayer soft lithography.[8,18] The top fluidic layer included a circulation loop (3.2 mm in diameter) that communicated with five pairs of inlet and outlet channels (100 μm wide, 10 μm deep). Holes (600 μm in diameter) punched at each end of the inlet (I1–I5) and outlet (O1–O5) channels were connected to syringe pumps by small metal tubes and Tygon tubing. The bottom pneumatic layer consisted of ten microchannels (50 μm wide, 10 μm deep) and five sets of individual microvalves and twin microvalves ($300 \times 200\ \mu m^2$). The five microvalves (valves 0, 2, 4, 6, and 8) located on the circulation loop were used to segment the circular mixer into five equal parts and also worked as a peristaltic pump to mix the different fluids, whereas the five twin microvalves (valves 1, 3, 5, 7, and 9) located on the inlet and outlet microchannels were used to control the flow of fluids. The overall size of the microfluidic device was 22.62 mm long and 22.2 mm wide.

As PDMS is gas-permeable, the valves were initially filled with water to prevent the diffusion of air bubbles through the PDMS membrane into the top fluidic channels. The valves were operated pneumatically by controlling the flow of N_2 gas into the valves. The valves on the bottom layer were pressed up against the microchannels on the top layer, which resulted in a blockage of flow. When a series of on/off actuation sequences was applied, the solutions in the circular mixer

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[**] This work was supported by the SRC program of the Korea Science and Engineering Foundation (KOSEF) (R11-2005-008-02001-0), the KOSEF grant (NRL) funded by the Korean government (MOST) (R04-2007-000-2007-0), Seoul R&BD Program (108/6), and BK21. S.K. was supported by an Ewha Exchange Scholarship.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

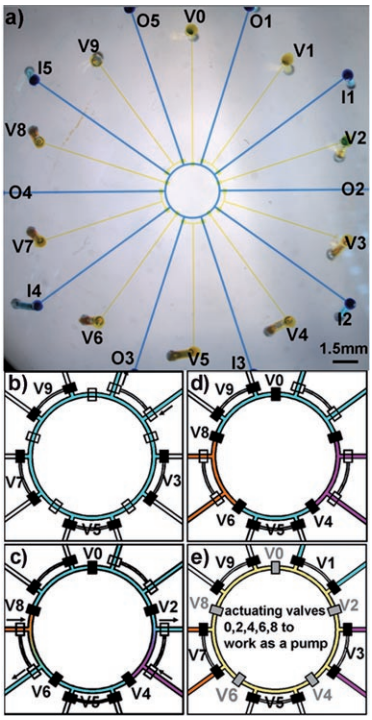
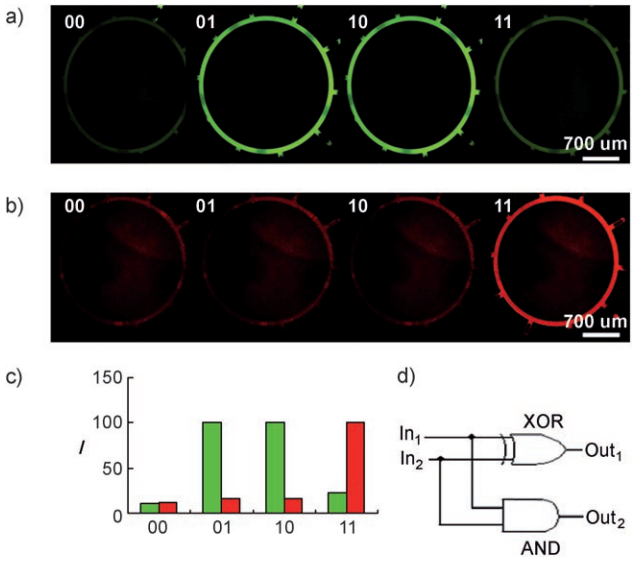


Figure 1. Image of a logic gate microfluidic system and schematic diagrams of the liquid mixing processes in the chip. a) The bottom pneumatic layer was visualized by filling it with yellow food dye, whereas the top fluidic-channel layer was filled with blue dye. The fluidic inlets and outlets are annotated as I1, inlet 1 and O1, outlet 1; the pneumatic inlets are numbered V0 to V9. b) Chemosensor (cyan) is introduced into the circulation loop through inlet 1. Closed valves are annotated as V3, valve 3. c, d) HCl (orange) and NaOH (magenta) are introduced into two parts of the circulation loop and displace the chemosensor. e) The three different liquids are thoroughly mixed by peristaltically actuating valves 0, 2, 4, 6, and 8. (□ Open valves, ■ closed valves, ■ actuating valves in peristaltic pumping mode).

were pumped round, thus resulting in a mixing process (Figure 1).

Figure 1 b–e illustrates the operation of the chip. All the valves were closed before loading the reagents. A fluorescent chemosensor solution was first loaded in the chip by opening valves 0, 1, 2, 4, 6, and 8. Once the loop (Figure 1 b) was filled with the chemosensor solution, the special pH-conditioning solutions (pH 2 or pH 12 buffer) or metal-ion solution (Cu^{2+}) were loaded from the inlets (I2 and I4) and the existing chemosensor was pushed out of the outlets (O2 and O4) by closing valves 0, 2, 4, 6, and 8 and opening the twin valves (3 and 7) simultaneously (Figure 1 c). For example, valves 3 and 7 were opened to load the pH 2 and pH 12 buffers, respectively (Figure 1 c and d). After closing valves 1, 3, and 7 and opening valves 0, 2, 4, 6, and 8, the three different liquids (chemosensor, pH 2 and pH 7 buffers) were merged in the circulation loop channel (Figure 1 e). As a result, the chemosensor solution took three parts of the loop while the input solutions took the remaining two parts. By operating valves 0, 2, 4, 6, and 8 in peristaltic pumping mode, the liquids were mixed thoroughly within 3 min (Figure 1 e). The circulation speed could be adjusted by controlling the pumping frequency, and the direction of rotation could be changed by simply reversing the pumping sequence. After complete mixing, the fluorescence intensity (measured using the Image J program) in the circulation loop was observed by fluorescence microscopy.

Fluorescein derivative **1** was used for the XOR gate in the microfluidic device (Figure 2 d). As shown in Figure 2 a, compound **1** showed a strong green fluorescence emission



e)

input		output	
In_1 H^+	In_2 H^+	XOR/Out_1 green (*I*)	AND/Out_2 red (*I*)
0	0	0 (low, 10.4)	0 (low, 12.1)
0	1	1 (high, 100)	0 (low 16.2)
1	0	1 (high, 100)	0 (low, 16.2)
1	1	0 (low, (21.6)	1 (high, 100)

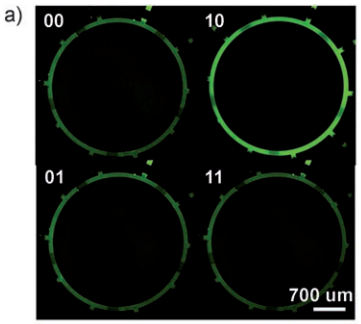
Figure 2. A half-adder molecular logic gate composed of an XOR gate (**1**, green) and an AND gate (**3**, red). a) Fluorescence images of **1** (green) in the presence of two H^+ inputs, b) fluorescence images of **3** (red) in the presence of two H^+ inputs, c) fluorescence intensities, d) a half-adder circuit, and e) a truth table of XOR and AND logic gates.

($\lambda_{max} = 525$ nm) in the neutral range with fluorescence quenching effects being observed at acidic and basic pH. The fluorescence quenching effect of compound **1** at basic pH can be explained by a photoinduced electron-transfer (PET) mechanism from the benzylic amine.[2a] On the other hand, the fluorescence quenching effect in the acidic region can be attributed to the formation of the fluorescein cation.[3l]

As shown in Figure 2 b, rhodamine B derivative **3** displayed a strong red fluorescence emission at acidic pH and fluorescence quenching effects at neutral and basic pH, which resulted in an AND logic gate (Figure 2 d). Rhodamine B derivatives exhibit fluorescence enhancement upon the addition of metal ions or protons, in which the spirolactam (nonfluorescent) to ring-opened amide (fluorescent) process was utilized.[19] Addition is carried out by a molecular half adder using an XOR logic gate to generate the sum digit and

an AND gate to produce the carry digit (Figure 2e). This is the first time a half-adder molecular logic gate has been demonstrated in a microfluidic device. Mixing of these two sensors (**1** and **3**) for a half-adder logic gate in a single microfluidic system was also performed successfully (Figure S1 in the Supporting Information).

Fluorescein derivative **2** was also reported to show a large fluorescence quenching effect with Cu^{2+} ions in the nanomolar range.[16] Operation by Cu^{2+} and OH^- demonstrated an INHIBIT gate (Figure 3). At neutral pH, compound **2** showed relatively strong fluorescence and the addition of Cu^{2+} or/and OH^- induced large fluorescence quenching effects. The fluorescence quenching effect at basic pH can be explained by a similar PET mechanism, as described above.

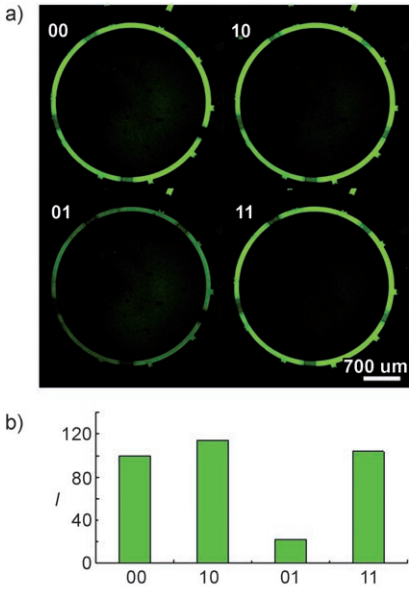


c)

input		output	
OH^-	Cu^{2+}	INH (*I*)	
0	0	low (23.60)	0
1	0	high (100)	1
0	1	low (21.59)	0
1	1	low (19.50)	0

Figure 3. a) Fluorescence images of **2** (10 μM) as an INHIBIT (INH) logic gate at pH 7, b) changes in the fluorescence intensity of **2** in the presence of OH^- (pH 12) and Cu^{2+} (100 μM), and c) a truth table of the INH logic gate.

Copper-binding studies in transferrin (Tf) have been reported because copper is known to play an important role in a number of neurodegenerative diseases, such as Alzheimer's and Wilson's diseases.[20] As shown in Figure 4, the microfluidic system with fluorescent chemosensor **2** as sensor molecule was used to monitor the uptake of Cu^{2+} ions by copper-binding proteins, such as Tf. This tendency arose as a result of the regeneration of the fluorescence of fluorescein derivative **2** because of Cu^{2+} uptake by Tf instead of **2**. The inverse output (or negative logic gate) result can be used as an INHIBIT logic gate (Figure 4c). To the best of our knowledge, this is the first example of a molecular logic gate utilizing a protein as the input. This particular microfluidic system has potential as a lab-on-a-chip-type sensor for monitoring the Cu^{2+} ion uptake by copper-binding proteins.



c)

input		output
Tf	Cu^{2+}	positive logic
0	0	**1** (high, 100)
1	0	**1** (high, 114.4)
0	1	**0** (low, 21.3)
1	1	**1** (high, 103.9)

Figure 4. a) Fluorescence images of **2** (10 μM) in the presence of Cu^{2+} and Tf, b) fluorescence intensities, and c) a truth table of the INH logic gate.

In conclusion, we have demonstrated for the first time a molecular logic gate in a microfluidic device. In particular, a combinatorial circuit, such as a half adder, was demonstrated by utilizing a fluorescein derivative (**1**) and a rhodamine B derivative (**3**). Furthermore, the first example of a molecular logic gate using a protein and Cu^{2+} ions as the two inputs of an INHIBIT gate was shown, in which a Cu^{2+}-selective fluorescein derivative (**2**) was used as the communicating signal material. Molecular logic gates in microfluidic systems offer reduced reagent consumption, high throughput, and unprecedented automation. Therefore, this new approach can be considered important progress towards a molecular computing system.

Received: August 20, 2007
Published online: October 17, 2007

Keywords: chemosensors · fluorescence · logic gates · microfluidics · molecular devices

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