



Supporting Information

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Fluorescent Molecular Logic Gates using Microfluidic Devices

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Experimental Section

General methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel 60 (230-400 mesh ASTM, Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was carried out using Merck 60 F₂₅₄ plates with a thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F₂₅₄ plates with a thickness of 1 mm. Melting points were measured using a Büchi 530 (Büchi Labortechnik AG, Flawil, Switzerland) melting point apparatus, and are uncorrected. ¹H NMR and ¹³C NMR spectra were recorded using Bruker 250 (ScotCHEM, Scotland, UK) or Varian 500 (The Burnham Institute, CA, USA). Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL Co., Tokyo, Japan). UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer (Kontron Instruments Ltd., Milan, Italy). Fluorescence emission spectra were obtained using RF-5301/PC spectrofluorophotometer (Shimadzu, Kyoto, Japan). SU-8 2007 was purchased from MicroChem Corp. (Newton, MA, USA), PDMS (polydimethyl siloxane) prepolymer (Sylgard 184A and 184B) were purchased from Dow Corning (Midland, MI, USA), and silicon wafers were purchased from Single Crystal Substrates of America, Inc (IL, USA). Transferrin was purchased as an iron free form from Sigma-Aldrich Inc. (St. Louis, MO, USA) Compounds **1**, **2**¹ and **3**² were synthesized following the reported procedures.

Compound 1. Dimethylamine (0.9 mL, 7.97 mmol) and paraformaldehyde (0.22 g, 7.47 mmol) were combined in 20 mL of CH₃CN and refluxed for 30 min. 2,7-Dichlorofluorescein (1.00 g, 2.49 mmol) in 30 mL of CH₃CN/H₂O (1:1) was added to the solution and the reaction mixture was refluxed for 24 h. The solvent was removed completely under vacuum. The product was dissolved in 20 mL of ethanol and kept in deep fridge for 3 days. Precipitate obtained was filtered on frit, washed thoroughly with spirit and dried under vacuum to give a deep red colored solid (0.2 g, 15.6%): mp 162-165 °C; ¹H NMR (250 MHz, CDCl₃) δ 7.68 (d, 1H, *J* = 7.6 Hz), 7.48 (t, 1H, *J* = 7.4 Hz), 7.21 (t, 1H, *J* = 7.4 Hz), 7.14 (s, 2H), 6.63 (d, 1H, *J* = 7.6 Hz), 4.39 (s, 4H), 2.78 (s, 12H); ¹³C NMR (62.5 MHz, CDCl₃) δ 173.8, 158.6, 154.9, 139.4, 130.3, 130.2, 129.6, 129.2, 128.8, 127.5, 110.8, 104.7, 57.3, 52.2, 42.6; FAB Mass *m/z* = 541.0903 (M+Na)⁺, calc. for C₂₆H₂₄Cl₂N₂O₅Na = 541.0901.

Fabrication of SU-8 masters. Mask designs were created in AutoCAD (San Rafael, CA, USA) and printed on high-resolution transparency films (Han & All Tech., Seoul, Korea). The dimensions of the fluidic channels were 100 μm × 10 μm (*w* × *h*) while the pneumatic control channels were 50 μm × 12.5 μm (*w* × *h*). The pneumatic valves were 200 μm × 300 μm (*w* × *l*). Masters for the valves and fluidic channels (valve

¹ J. Jun, J. - A. Kim, K. M. K. Swamy, S. Park, J. Yoon, *Tetrahedron Lett.* **2006**, *47*, 1051.

² J. H. Soh, K. M. K. Swamy, S. K. Kim, S. Kim, S. -H. Lee, J. Yoon, *Tetrahedron Lett.* **2007**, *48*, 5966.

master and channel master) were made by spinning a thin layer ($\sim 10 \mu\text{m}$) of negative photoresist SU-8 2007 (500 rpm, 5 s; 1500 rpm, 30 s) on a separate 4-in-diameter silicon wafer and soft baking them in two steps on a hotplate (65°C, 1 min; 95°C, 2 min). After cooling, the photoresist was exposed through a transparency mask for 30s in a exposure & mask alignment system (MDA-400, Midas System Co., Daejeon, Korea) with a 350 watt UV light and postbaked (65°C, 1 min; 95°C, 2 min). After being developed, the wafers were then hard baked (150°C, 15 min) to reflow the photoresist to form curved features. Then molds were treated with trimethylchlorosilane (United Chemical Technologies, Inc., Bristol, PA, USA) vapor for 15 min to facilitate mold release.

Fabrication of PDMS microfluidic Devices. PDMS devices with valves were formed by multilayer soft lithography. A two component silicone elastomer (Sylgard 184 mixed in a ratio of 10 A : 1 B) was poured on the channel mold to a thickness of $\sim 4 \text{ mm}$, while elastomer (30 A : 1 B) was spun onto the valve master with a thickness of $\sim 40 \mu\text{m}$ (500 rpm, 5 s; 2000 rpm, 30 s). To perform bonding, each layer was separately baked at 80°C for 30 min. The thick layer was then peeled off the mold and holes for inlets and outlets were punched by a hand punch (Technical Innovation Inc., Brazoria, TX, USA) for connection to syringe. After cleaning, it was aligned over the valve mold under a microscope. The two-layer structure was then baked at 80°C overnight, chemically cross-linking the two layers into a single structure. This bonded PDMS layer was peeled from the layer master and holes were punched for connection to an external pressure controller. The PDMS was treated with oxygen plasma at 50 watt for 30 s and bonded to a glass slide to form an enclosed fluidic system.

System setup and pneumatic control. The device was mounted on an inverted microscope (Nikon Eclipse TE 2000-U, Tokyo, Japan). Fluorescence excitation was provided by a mercury lamp (100 W). Two filter sets were used. One consisted of an EX 510-560 excitation filter, DM 575 beam splitter and BA 590 barrier filter, the other consisted of an EX 450-490 excitation filter, DM 505 beam splitter and BA 520 barrier filter. The image was recorded by using a SPOT insight digital camera (Diagnostic Instruments, Inc., MI, USA). Each actuation line on the chip was connected with a stainless steel pin (New England Small Tube, Litchfield, NH, USA) and polyethylene tubing (TYGON®, Saint-Gobain PPL Co., Cleveland, OH, USA) to an external solenoid valve (Lee Company, Westbrook, CT, USA) controlled by a digital data I/O card (PCI-DIO-32HS, National Instruments Co., Austin, TX, USA). Valves were pressurized by nitrogen gas gated by solenoid valves driven by a computer running LABVIEW (National instruments Co., Austin, TX, USA). The control channels were initially filled with water to prevent diffusion of air bubbles through the PDMS membrane into the flow layer. The control pressure was maintained at 30 psig. Fluorescence intensity was measured by the Image J program (NIH, Bethesda, MD, USA) and data was evaluated by Excel.

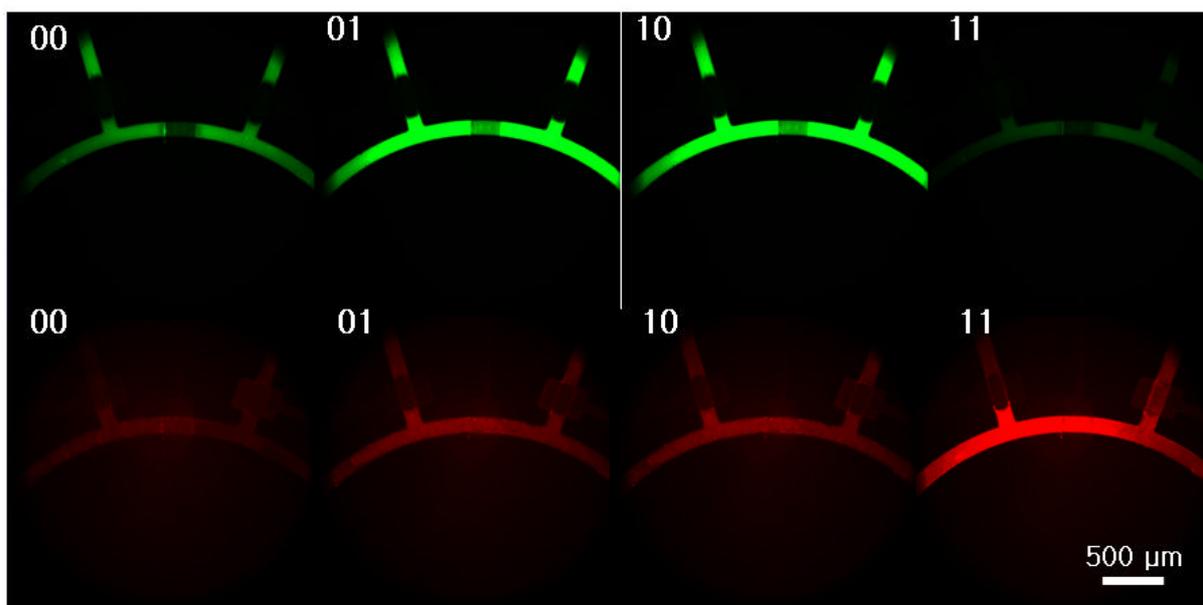


Figure-S1. Fluorescence images of 5 μM **1** (green) and 5 μM **3** (red) mixture in the present of two H^+ (pH=2) inputs in one microfluidic system (Images were recorded with changes between filter sets as well as a digital camera system.^a)
^a A half-adder molecular logic gate was obtained as described in Figure 3.

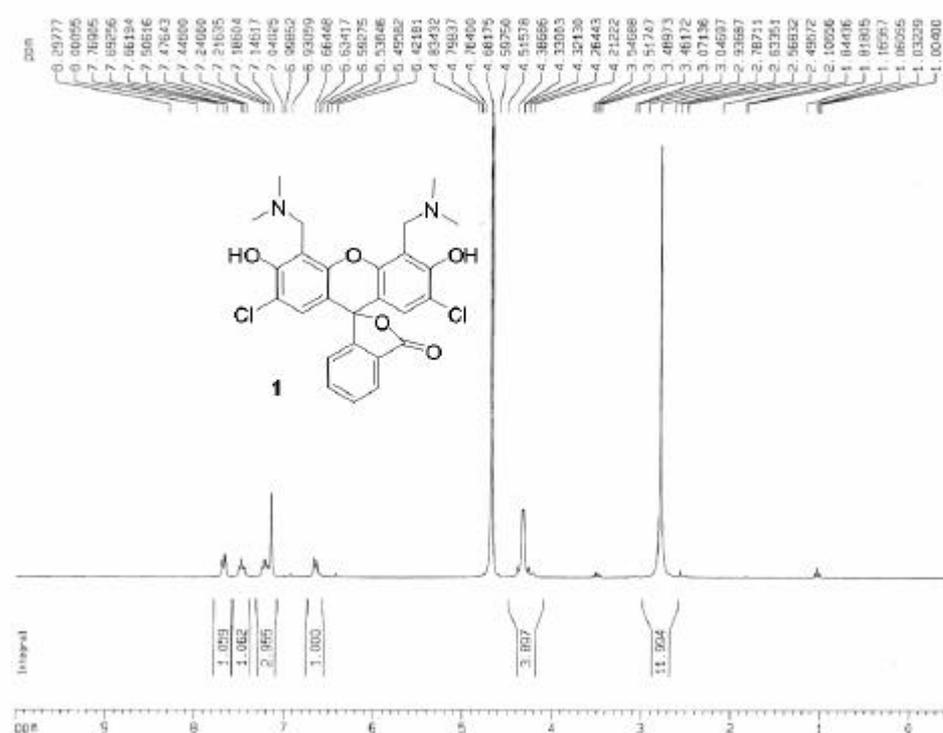


Figure S2. ^1H NMR (250 MHz) of compound **2** in CD_3OD .

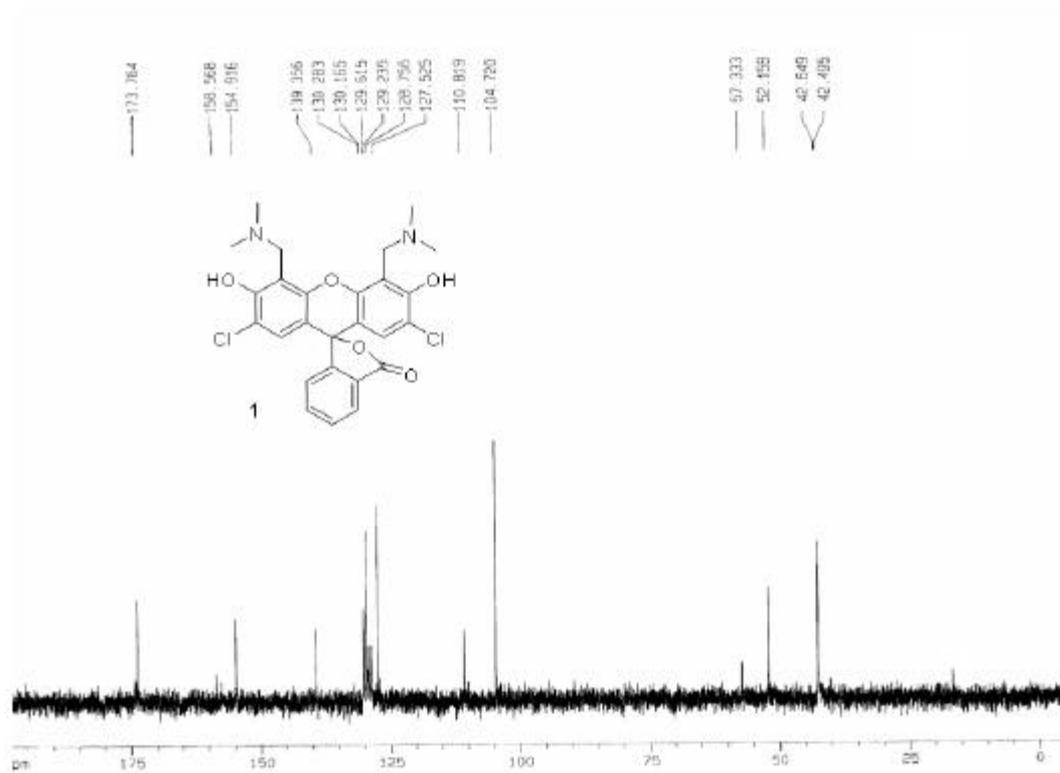


Figure S3. ^{13}C NMR (62.5 MHz) of compound 2 in CD_3OD .