Electronic Supplementary Material (ESI) for Chemical Communications.
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Enzyme-Based Logic Gates Switchable Between OR, NXOR and NAND Boolean Operations Realized in a Flow System

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Electronic Supplementary Information

Experimental Section

Chemicals and Materials

Glucose-6-phosphate dehydrogenase (G6PDH; E. C. 1.1.1.49) from *Leuconostoc mesenteroides*, glucose dehydrogenase (GDH; E.C. 1.1.1.47) from *Pseudomonas sp.*, lactate dehydrogenase (LDH; E. C. 1.1.1.27) from porcine heart, malate dehydrogenase (MDH; E.C. 1.1.1.37) from porcine heart, pepsin (E. C. 232.629.3) from porcine gastric mucosa, β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), β-nicotinamide adenine dinucleotide hydrate (NAD^+), glucose-6-phosphate (G6P), D-(+)-glucose (Glc), pyruvate (Pyr), oxaloacetic acid (Oxacet), glutaric dialdehyde, poly(ethyleneimine) solution (PEI) (average M_w ca. 750,000), 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris-buffer) and other standard inorganic/organic reactants were purchased from Sigma-Aldrich and used as supplied. Ultrapure water (18.2 MΩ·cm) from NANOpure Diamond (Barnstead) source was used in all of the experiments.

Instruments and Devices

Flow cells (μ-Slide III 3in1 Flow Kit; ibidi GmbH), Figures ESI-1 and ESI-2, were used for the biocatalytic reactions. A Shimadzu UV-2450 UV–Vis spectrophotometer with flow-through quartz cuvettes (1 cm optical pathway) connected to the tubing of the flow device was used for all optical measurements. The reacting solutions were pumped through the flow cells and spectrophotometer cuvettes with the help of a peristaltic pump (Gilson Minipuls 3) connected with polyethylene tubing, 1 mm internal diameter, Figures ESI-3 and ESI-4.

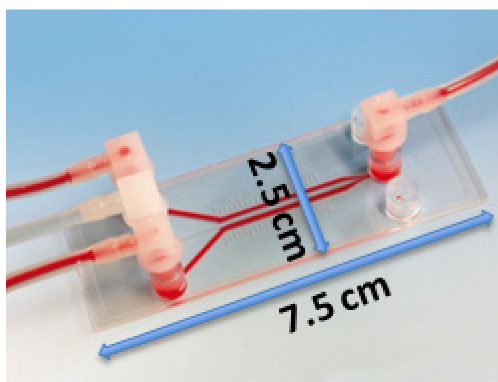

Figure ESI-1: The flow cell used in the experiments. The flow channels are filled with a red color solution to visualize them. The internal total channel volume of the flow cell is 60 μL, with the height of all channels being 0.4 mm and the width of the channels thin/thick being 1mm and 3 mm respectfully. Adopted from the company web-site with the permission:
http://www.e-shop.vitaris.com/epages/Vitaris.sf/en_GB/?ObjectPath=/Shops/Vitaris/Products/81314-IBI.

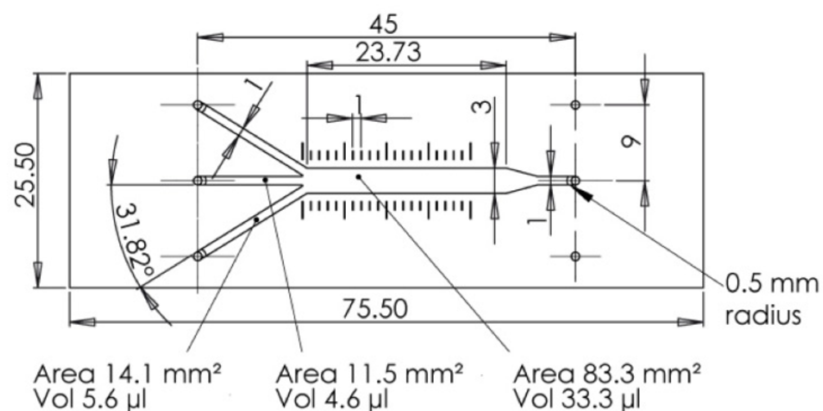

Figure ESI-2: The flow cell used in the experiments, all measurements are in millimeters. The internal total channel volume of the flow cell is 60 µL, with the height of all channels being 0.4 mm and the width of the channels thin/thick being 1 mm and 3 mm, respectfully. Adopted from the company web-site with the permission: http://www.autom8.com/biomicro_SlideIII_3in1.html.

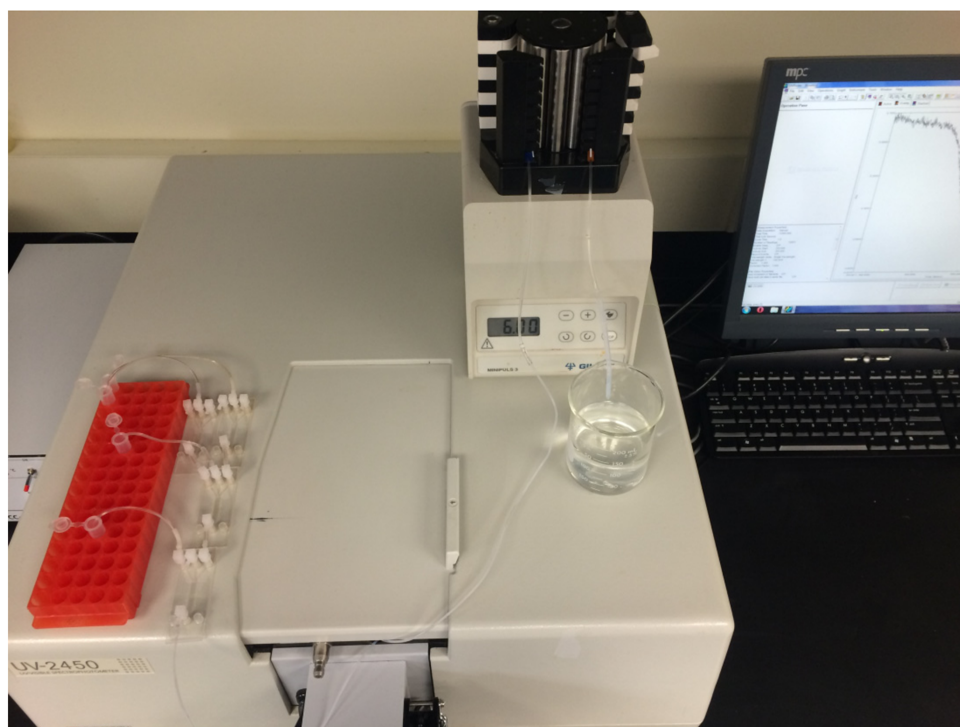

Figure ESI-3: The experimental setup. The photo shows the flow cells, spectrophotometer and peristaltic pump.

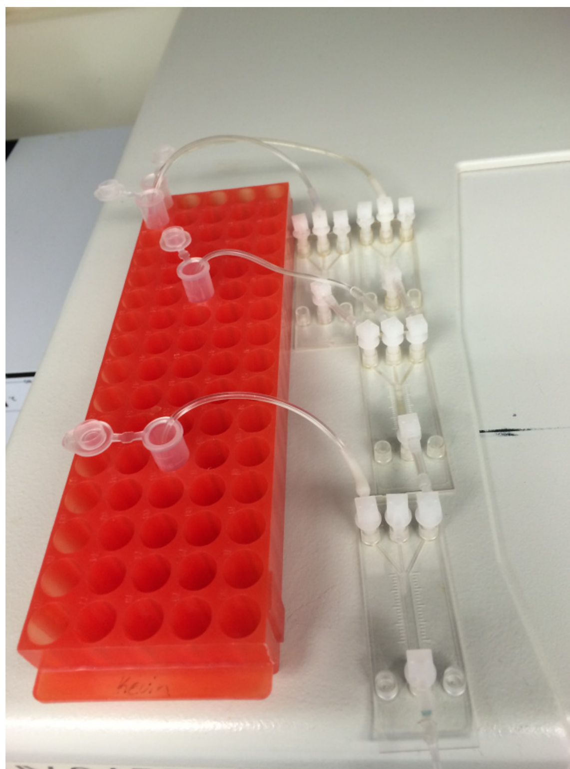

Figure ESI-4: The experimental setup. The photo shows the flow cells and Eppendorf tubes with the stock solutions.

Immobilization of Enzymes in the Flow Cells

Before any experimental data were realized, the flow cells were thoroughly cleaned with concentrated sulfuric acid to remove any residual physical adsorption of PEI left on the cell surface from previous experiments. After this initial preparatory step, all subsequent cleanings were conducted with the following method. The flow cells were washed with a minimum of 10 mL of deionized water and then reacted with pepsin solution, 0.1 mg/mL, in 0.1 M phosphate buffer, pH 2.0, for 1 hour. Then, the cells were washed with a minimum of 10 mL of deionized water. These cleaning steps aimed at removing remnant enzymes from previous experiments and prepared the cell surface for adsorption of PEI. Then, the flow cells were treated with a PEI solution (4% v/v) for 1 h and then, thoroughly washed with 5 mL of deionized water, resulting in physical adsorption of PEI on the internal microscopy plastic surface and providing amino groups for the enzyme immobilization. The amino-functionalized surface was then reacted with glutaric dialdehyde (10% v/v) for 1 h; after that, the surface was washed with 5 mL of deionized water to remove non-reacted glutaric dialdehyde. The total volume of the enzyme solutions (GDH ca. 140 units/mL; G6PDH ca. 280 units/mL; LDH ca. 343 units/mL; MDH ca. 300 units/mL) was set at 75 µL to closely match the total channel volume. These enzyme solutions were then reacted with the flow cells activated with glutaric dialdehyde for 1.5 h. Following this, the cells were thoroughly washed with Tris-buffer (0.1 M, pH 7.4) to remove any non-reacted enzymes from the cells. The procedure resulted in the enzyme covalent binding to the adsorbed PEI through Schiff-base bonds. The flow cell device with the immobilized enzymes demonstrated reproducible performance for at least two days allowing pumping of the input solutions over long period of time, thus proving stable immobilization of the enzymes.

Optimization of the Input Concentrations

The input concentrations (Glc, G6P, Pyr and Oxacet) were optimized for the specific enzyme activity in their respective flow cells. The optimization was aimed at the output signals with the comparable intensity upon the application of different combinations of input signals.

Flow Cell Performance and the Output Signal Measurements

The input signals (represented with Glc, 0.3 mM; G6P, 0.86 mM; Pyr, 0.065 mM and Oxacet, 0.125 mM solutions) also containing NADH, 0.375 mM, and NAD^+, 2.75 mM) were pumped through the flow system with the volumetric rate of 50 µL/min. The input concentrations were calculated as they appeared in the reacting flow cells after dilution with all other pumped solutions. Optical absorbance measurements were performed at λ = 340 nm characteristic of NADH. The reference channel (cuvette) of the spectrophotometer was filled with the background ("machinery") solution containing NADH (0.375 mM) and NAD^+ (2.75 mM), thus allowing the absorbance change measurements vs. the composition of the background solution.