Enzyme-Based Reversible Logic Gate Realized in a Flow System

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Electronic supplementary information (ESI)

Chemicals and materials:
Alkaline phosphatase (AP; E. C. 3.1.3.1) from bovine intestinal mucosa, glucose-6-phosphate dehydrogenase (G6PDH; E. C. 1.1.1.49) from *Leuconostoc mesenteroides*, lactate dehydrogenase (LDH; E. C. 1.1.1.27) 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), pyruvate (Pyr), p-nitrophenyl phosphate (PNPP), glutaric dialdehyde, poly(ethyleneimine) solution (PEI) (average M_w ca. 1300), 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), Figure ESI-1, 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, Figure ESI-2.
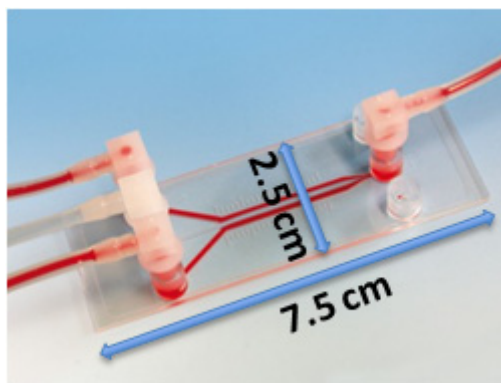

Figure ESI-1: The flow cell used in the experiments.

Figure ESI-2: The experimental setup. The photo shows the flow cells, spectrophotometer and peristaltic pump.

Immobilization of enzymes in the flow cells:

The flow cells were cleaned and prepared for further modification in the following way. They were washed with deionized water 5 times and then reacted with pepsin solution, 0.5 mg/mL, in 0.1 M phosphate buffer, pH 2.0, for 1 hour. Then, the cells were washed with deionized water again 5 times. 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 (2% v/v) for 1 h and then, thoroughly washed with water 5 times, resulting in physical adsorption of PEI on polystyrene and providing amino groups for the enzyme immobilization. Then, the amino-functionalized surface was reacted with glutaraic dialdehyde (5% v/v) for 1 h; after that, the surface was washed with water 5 times to remove non-reacted glutaric dialdehyde. The enzyme solutions (AP ca. 500 units/mL; G6PDH ca. 280 units/mL; LDH ca. 600 units/mL) were reacted with the flow cells activated with glutaric dialdehyde for 1 h and then, the cell were thoroughly washed with Tris-buffer (0.1 M, pH 7.1) to remove 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 (PNPP, Pyr, G6P) were optimized for the specific enzyme activity in the flow cells. The optimization was aimed at the output signals with the comparable intensity upon application of different combinations of the input signals.

Flow cell performance and the output signal measurements:
The input signals (represented with PNPP, Pyr, G6P solutions also containing NADH, 0.4 mM, and NAD^+, 10 mM) were pumped through the flow system with the volumetric rate of 50 µL/min. Optical absorbance measurements were performed for the Identity gate channel (Output A) at $\lambda = 420$ nm characteristic of PNP and for the XOR gate channel (Output B) at $\lambda = 340$ nm characteristic of NADH. The reference channel (cuvette) of the spectrophotometer was filled with the background ("machinery") solution containing NADH (0.4 mM) and NAD^+ (10 mM), thus allowing the absorbance change measurements vs. the composition of the background solution.