Analyst

COMMUNICATION

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View Journal | View Issue

An enzyme-based reversible CNOT logic gate realized in a flow system†

Cite this: *Analyst*, 2014, **139**, 1839

Received 18th January 2014
Accepted 27th January 2014

DOI: 10.1039/c4an00133h

www.rsc.org/analyst

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An enzyme system organized in a flow device was used to mimic a reversible Controlled NOT (CNOT) gate with two input and two output signals. Reversible conversion of NAD^+ and NADH cofactors was used to perform a XOR logic operation, while biocatalytic hydrolysis of *p*-nitrophenyl phosphate resulted in an Identity operation working in parallel. The first biomolecular realization of a CNOT gate is promising for integration into complex biomolecular networks and future biosensor/biomedical applications.

Recent research in unconventional computing,[1] particularly realized using molecular[2–4] and biomolecular[5–7] systems, has received much attention and resulted in artificial (bio)chemical systems mimicking Boolean logic operations, including AND, OR, XOR, NAND, NOR and other logic gates.[3,4,7] Sophisticated molecular designs have allowed reversible,[8–12] reconfigurable[13] and resettable[14,15] logic gates for processing chemical information. Further sophistication of the systems has resulted in the assembly of logic gates in cascades performing complex computing functions mimicking electronic circuitries,[16–19] and performing elementary computing.[20,21] Achieving versatile and multi-functional information processing with biomolecules in the systems, offering new functionalities supplementary to electronics, has been the primary challenge in the field.[5–7,22] Particular attention was given to interfacing of the biomolecular information processing systems with electronic devices.[23–25] For multi-step, multi-signal information processing of increased levels of complexity, binary gates have to be connected in networks.[7,18] This requires development of paradigms for scalability and avoidance of noise buildup,[22] as well as "clocking" (temporal control) and ultimately spatial separation of the various steps[26] of multistage biochemical processes.

Molecular[8–11] and biomolecular[12,27,28] systems mimicking operation of reversible logic gates (*e.g.*, Toffoli,[12] Fredkin[12,27,28] and Feynman[9,10] gates) are of particular interest, specifically for (bio)sensor applications, since they provide unique output patterns for each combination of input signals. (Note that in this context "*reversibility*" has a different meaning than in chemistry; in information processing "*logical reversibility*" means the possibility to recover initial information from the processed information.) While justifying the importance of reversible logic gates, there is a common misconception about the physical meaning of reversibility in information processing systems. Upon irreversible logic computations, each bit of information lost generates $kT \ln 2$ Joules of heat energy based on thermodynamic consideration.[29] Theoretically and experimentally, in some electronic realizations, reversible information processing[30,31] should not be accompanied by entropy increase,[32] thus being an energy saving process. However, this is not applicable to chemical systems where energy dissipation is inevitable. Therefore, the molecular/biomolecular realizations of reversible logic gates keep only *logic* reversibility, while the energy savings are illusory goals. This rather trivial and obvious conclusion should be remembered when reversible logic gates are attempted in (bio)molecular systems.[12] Still *logic* reversibility, which means the possibility to derive the original input pattern from each unique output signal combination is an important feature of the (bio)molecular information processing. In order to be practically useful, reversible logic gates should be integrated into complex information processing networks. Unfortunately, most of the experimentally realized systems were all-photonic, where the input/output signals were optical signals which cannot be combined with molecular networks.[8–11] Only a few experimental examples have demonstrated reversible logic operation with input/output signals represented by biomolecules (usually DNA),[12,28] thus allowing

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† Electronic supplementary information (ESI) available: Experimental details on the enzyme-system operation. See DOI: 10.1039/c4an00133h

extension of information processing and integration of reversible logic gates into biomolecular networks mimicking biological systems. Recently designed logic networks based on enzymatic cascades[7,18,19] are the easiest for the practical realization of systems where logic operations are particularly useful for biomedical sensing and diagnostic applications.[33] Despite the fact that complex multi-input/multi-step information processing systems have been successfully realized with enzymatic cascades,[7] the requirement of several (at least two) independently read output signals for realization of reversible logic gates is not a simple task. Cross-talking between enzymatic pathways and chemically produced output signals limit the complexity of the enzyme-based logic systems when they are realized in a homogeneous system. In order to assemble more complex systems for realization of reversible logic gates, "clocking" (temporal control) and spatial separation of various steps (*e.g.*, in flow devices) are needed.[34,35] The present paper reports on the first realization of a reversible logic gate with enzyme cascades performed in a flow system, where the chemical input and output signals can be potentially extended to additional information processing steps, thus allowing further increase of the system complexity.

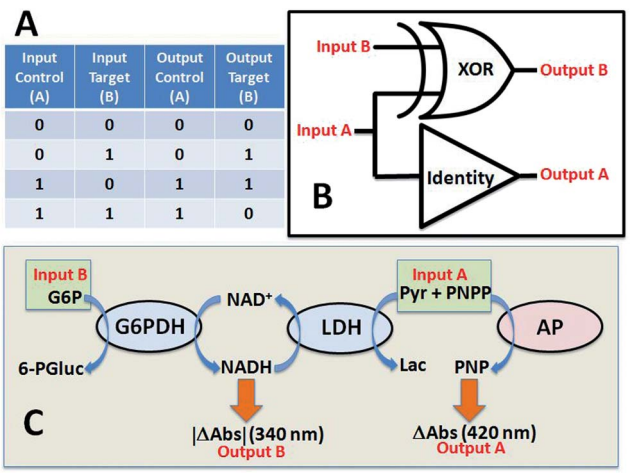
We realized a Controlled NOT (CNOT) gate, which is the only non-trivial reversible gate with two input and two output signals.[36] The first experimental realization of a CNOT gate was accomplished in 1995.[37] From that time the CNOT gate has been implemented in many physical systems demonstrating great importance for future quantum computing,[38] however, it has never been researched in chemical, particularly in biochemical, systems aiming at its integration with biomolecular logic networks. Scheme 1(A) shows the truth table of the CNOT gate. The CNOT gate flips the second Input B (the target input) and directs it to the second Output B (the target output) if and only if the first Input A (the control input) is 1, while the first Input A is always copied to Output A (the control output). In other words, Output A represents an Identity gate for Input A, while Output B processes Inputs A and B according to an XOR logic operation. The logic scheme illustrating CNOT operation is shown in Scheme 1(B), where Input A is directed to an Identity gate and a XOR gate is activated by both Input A and Input B. In our experimental realization (see experimental details in the ESI†) we defined Input A as a Tris-buffer solution (0.1 M, pH 7.1) containing *p*-nitrophenyl phosphate (PNPP) and pyruvate (Pyr) with concentrations 10 mM and 1 mM, respectively, to represent logic 1 value. Logic 0 value for Input A was defined as the absence of PNPP and Pyr in the solution. Input B was defined as a Tris-buffer solution (0.1 M, pH 7.1) containing glucose-6-phosphate (G6P), 6 mM, for logic 1 value. The absence of G6P was used to encode logic 0 for Input B. Note that the concentrations of the reacting species were optimized experimentally as will be described later. PNPP representing one of the reacting species in Input A was converted to *p*-nitrophenol (PNP) in the reaction biocatalyzed by alkaline phosphatase (AP; E. C. 3.1.3.1) resulting in the optical absorbance increase (λ_{max} = 420 nm), Scheme 1(C). This biocatalytic reaction represented the Identity gate, Scheme 1(B), and the produced absolute values of absorbance changes were used as Output A, which was defined as logic 1 or 0 when $|\Delta Abs| > 0.1$ or $|\Delta Abs| < 0.1$, respectively. Input A was also directed to the biochemical system performing an XOR logic operation, Scheme 1(B).

We designed an XOR gate with two oppositely directed biocatalytic redox reactions[39] activated by pyruvate (Pyr; second reacting species in Input A) and glucose-6-phosphate (G6P; Input B), Scheme 1(C). Input B was defined as logic 0 and 1 for the absence of G6P and its presence with the concentration of 6 mM, respectively. The reaction media (all input solutions) also included NADH (0.4 mM) and NAD^+ (10 mM) cofactors as a part of the gate "machinery". NADH was oxidized by Pyr in the reaction biocatalyzed by lactate dehydrogenase (LDH; E. C. 1.1.1.27) producing NAD^+ and decreasing the NADH absorbance at λ_{max} = 340 nm. NAD^+ was reduced by G6P in the reaction biocatalyzed by glucose-6-phosphate dehydrogenase (G6PDH; E. C. 1.1.1.49) producing NADH and increasing its absorbance. Output B produced by the XOR gate, Scheme 1(B), was measured as absolute values of the absorbance changes at λ_{max} = 340 nm and defined as logic 1 and 0 for $|\Delta Abs| > 0.2$ or $|\Delta Abs| < 0.2$.

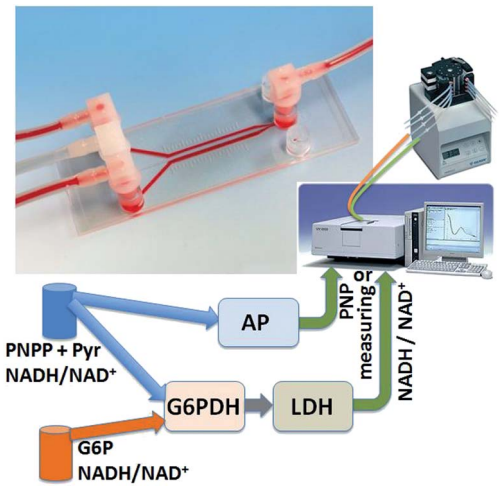
The Identity and XOR gates operating in parallel were realized in a flow system (μ-Slide III 3in1 Flow Kit; ibidi GmbH) outlined in Scheme 2. The solutions containing biochemicals representing Inputs A and B with the variable binary logic values 0 and 1, as well as the constant composition of the "machinery" represented by mixed NADH/NAD^+ were pumped through flow devices containing immobilized enzymes (see details in the ESI†) biocatalyzing chemical transformations mimicking logic operations. The flow design of the biochemical device allowed "clocking" (temporal control) and spatial separation of the reaction steps. While the Identity gate realized in a single flow unit performed a simple biocatalytic transformation of PNPP to PNP yielding an optically readable signal, the XOR gate was composed of two flow-through units: one modified with G6PDH that reduced NAD^+ when G6P was available; another modified with LDH that oxidized NADH in the presence of Pyr, Scheme 2. Therefore, the optical absorbance corresponding to the NADH

A

Input Control (A)	Input Target (B)	Output Control (A)	Output Target (B)
0	0	0	0
0	1	0	1
1	0	1	1
1	1	1	0



Scheme 1 (A and B) The truth table and logic circuitry for the CNOT gate, respectively. (C) The biocatalytic cascade mimicking the CNOT gate operation (abbreviations and reactions are explained in the text and technical details are given in the ESI†).



Scheme 2 Experimental realization of the biocatalytic CNOT gate in the flow device (abbreviations and processes are explained in the text and technical details are given in the ESI†).

concentration decreased in the presence of Pyr and absence of G6P (input combination 1, 0) and increased in the absence of Pyr and presence of G6P (input combination 0, 1). Since the NADH concentration and the corresponding absorbance were changed in different directions (decreasing and increasing), the Output B value 1 was defined as the absolute value of the absorbance change. The flow cells modified with G6PDH and LDH were optimized (balanced) in such a way that the simultaneous presence of Pyr and G6P (input combination 1, 1) resulted in no changes in the concentration of NADH, meaning logic value 0 for Output B. Balancing of the biocatalytic reaction rates was achieved by optimization of input concentrations for Pyr and G6P for the specific activity of the enzymes immobilized in the flow units. Obviously, the absence of Pyr and G6P (input combination 0, 0) did not result in any reaction and maintained the NADH absorbance resulting in logic 0 for Output B. These biocatalytic processes performed in the flow system allowed operation of the XOR part of the CNOT gate. It should be noted that the absorbance measurements for the solutions reacted in the flow device were performed *vs.* the "machinery" solution containing NADH/NAD^+ applied to the reference channel of the spectrophotometer, thus reflecting absorbance difference in the reacting solutions rather than their full absorbance.

Fig. 1 shows the experimental data obtained upon application of the input signals in different logic combinations. Fig. 1(A) shows the absorbance changes observed in Output A (from the Identity gate). In the absence of PNPP only minor absorbance changes were observed, meaning an Output A logic 0 value. Each time application of PNPP (regardless of the presence or absence of any other species) resulted in the absorbance increase at $\lambda_{max} = 420$ nm reflecting the formation of PNP and resulting in the output logic 1; thus, Input A was directly copied to Output A. Fig. 1(B) shows the XOR gate performance where only unbalanced input signals 0, 1 and 1, 0 resulted in the absorbance changes (output 1), while the absence of the reacting Pyr and G6P (inputs 0, 0) or their balanced application (inputs 1, 1) resulted in no absorbance changes (output 0).

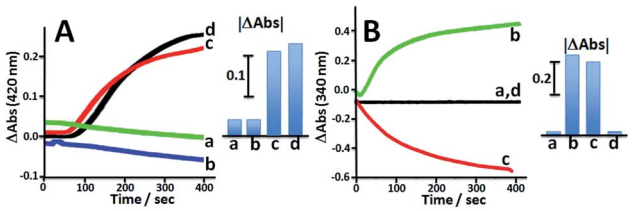


Fig. 1 (A and B) Optical responses of the Identity and XOR parts of the CNOT gate, respectively, upon application of input combinations: (a) 0, 0; (b) 0, 1; (c) 1, 0 and (d) 1, 1. The insets show the optical outputs obtained after 400 s of the flow system operation.

The first results for the CNOT gate realized in a biocatalytic flow system are promising for integrating the new reversible gate into complex biomolecular logic networks. The logic processing of biomolecular signals in the reversible mode will be particularly beneficial for biomedical/biosensing applications where each combination of the output signals corresponds to the unique pattern of the input signals, thus allowing restoration of the original input values. Application of logically reversible gates in the analysis of biomedically important biomarker signaling on various physiological dysfunctions, similarly to previously reported injury diagnostics,[40–43] is feasible. Technological realization of the information processing systems in fluidic devices allows "clocking" (temporal control) and spatial separation of the various steps of multistage biochemical processes, thus providing novel options for their sophistication and functional flexibility.

Acknowledgements

This work was supported by the National Science Foundation (award CBET-1066397).

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