Supporting Information

Bridging the Two Worlds: A Universal Interface between Enzymatic and DNA Computing Systems**

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Supporting Information

1. Chemicals and Reagents

Maltose phosphorylase (MPh; E.C. 2.4.1.8) from Enterococcus sp., hexokinase (HK; E.C. 2.7.1.1) from Saccharomyces cerevisiae, 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., alcohol dehydrogenase (AlcDH; E.C. 1.1.1.1) from Saccharomyces cerevisiae, sodium alginate from brown algae (medium viscosity, ≥2000 cP), pyrroloquinoline quinone (methoxatin disodium salt, PQQ), β-nicotinamide adenine dinucleotide sodium salt (NAD⁺), poly(ethyleneimine) solution (PEI), D-(+)-maltose monohydrate, adenosine 5’-triphosphate disodium salt hydrate (ATP), D-(+)-glucose, glucose-6-phosphate, poly-L-lysine hydrobromide (mol. wt. 30,000–70,000), (1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide (EDC), N-hydroxysuccimide (NHS), (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES-buffer), 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris-buffer), 3-(N-morpholino) propanesulfonic acid (MOPS-buffer) and other standard organic and inorganic materials and reactants were obtained from Sigma-Aldrich or J.T. Baker and used without further purification. Custom made DNA oligonucleotides, one labeled with FITC label, were purchased from Integrated DNA technologies (IDT); see the sequences and applied concentrations in the table below. All experiments were carried out in ultrapure water (18.2 MΩ-cm; Barnstead NANOpure Diamond).
Oligonucleotides used in the study:

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Conc., nM</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>F substrate</td>
<td>5'-AAG GT(dT-FAM) TCCT CG uCC CTG GGC A(BHQ-1)-3''</td>
<td>200</td>
<td>HPLC</td>
</tr>
<tr>
<td>F substrate mimic a</td>
<td>AAG GTTTGCC TCAGCC CTG GGC A</td>
<td>200</td>
<td>SD</td>
</tr>
<tr>
<td>3iANDa</td>
<td>5'-CCA GGG A GC CGA GCT AAG AC GTC TGCA CAC AAA TTC GGT TCT ACA GGG TA CGTTAGC</td>
<td>10</td>
<td>SD</td>
</tr>
<tr>
<td>3iANDb</td>
<td>5'-GTGAAGGG CAA ACA CCA TTG TCA CAC AAG GAT CCT TC A CAA CCA GAG GAA AC</td>
<td>10</td>
<td>SD</td>
</tr>
<tr>
<td>I1 (miR-122)</td>
<td>5'-TGG AGT GTG ACA ATG GTG TTG G</td>
<td>10</td>
<td>SD</td>
</tr>
<tr>
<td>I2 (miR-10b)</td>
<td>5'-TAC CCT GTA GAA CCG AAT TGG TG</td>
<td>10</td>
<td>SD</td>
</tr>
<tr>
<td>OP1</td>
<td>5'-TGC AGA C GT T GA AG G ATC CTC</td>
<td>0.8/5 c</td>
<td>SD</td>
</tr>
<tr>
<td>OP1-FITC e</td>
<td>FITC -5'-TGC AGA C GT T GA AG G ATC CTC</td>
<td>0.8/5 c</td>
<td>SD</td>
</tr>
</tbody>
</table>

a BHQ-1 – Black Hole Quencher 1;
b SD, standard desalting;
cca 0.8 and 5 nM concentrations correspond to the in situ generated OP1 concentrations served as logic values 0 and 1, respectively;
dThis oligonucleotide was only used in gel experiments (Figure S5) instead of F substrate.
eThe fluorescent labeled OP-1 derivative was used to estimate the concentrations of the in situ released oligonucleotide.

Nucleotides constituting the 10-23 catalytic core are in italics; nucleotides complementary to inputs I1 or I2 are underlined.

Scheme S1. Fluorescent signal-producing complex of 3iAND in the presence of the three oligonucleotide inputs.

2. Instrumentation

Fluorescent measurements were performed using a fluorescent spectrophotometer (Varian, Cary Eclipse). A Shimadzu UV-2450 UV–Vis spectrophotometer was used for optical absorbance measurements. Electrochemical experiments were performed using an electrochemical workstation (ECO Chemie Autolab PASTAT 10) and GPES 4.9 (General Purpose Electrochemical System) software. Potential measurements and control potential polymer deposition were performed using a Metrohm Ag/AgCl/KCl, 3 M, reference electrode.

3. Experimental procedures

*Alginate electrode functionalization.* Sodium alginate (1.5% w/w) was dissolved in 100 mM Na₂SO₄ (pH 6.0) aqueous solution and stirred for 30 min at 45 °C. The solution was cooled to room temperature, and FeSO₄ (35 mM) and oligonucleotide OP1 (2 µM) were added and mixed well. The alginate mixture was deposited on a graphite electrode (pencil rod, geometrical surface area ca. 0.57 cm²) upon oxidation of Fe²⁺ cations by applying a potential of +800 mV (vs. Ag/AgCl reference) for 60 seconds using a graphite counter electrode with a potentiostat and resulting in an Fe³⁺-cross-linked alginate matrix on a graphite surface containing the entrapped OP1 (Figure S2). The electrode was washed and allowed to incubate for 5 min in 0.25% (w/v) of poly-L-lysine prepared in 20 mM MOPS buffer, pH 7.4. The resulting electrode was washed thoroughly in water and then used...
in the release experiments. The detailed thickness analysis and electrochemical characterization of the alginate thin-film were reported elsewhere.\(^{[1]}\)

**Figure S1.** Illustration of different effect of Fe\(^{2+}\) and Fe\(^{3+}\) cations on alginate polymer: Fe\(^{3+}\) results in alginate cross-linking and gel formation, while Fe\(^{2+}\) does not formate gel keeping alginate in a soluble state. Note that these photos are shown for the illustration purpose only, since in the present work the alginate gel formation and dissolution were performed on an electrode surface (not in a bulk solution). Photographs represent the formation of alginate gel in the solution with Fe\(^{3+}\) cations composed of alginate (1.5% w/w), FeCl\(_3\) (35 mM) (right), while alginate solution with Fe\(^{2+}\) cations composed of alginate (1.5% w/w), FeSO\(_4\) (35 mM), and 0.1 M Na\(_2\)SO\(_4\) remains a viscous liquid (left). (Figure adopted from ref. 1c with permission).

**Figure S2.** Electrochemical deposition of the Fe\(^{3+}\)-cross-linked alginate film on a graphite electrode.
Functionalization of a graphite electrode with PQQ. A graphite electrode (pencil rod, geometrical surface area ca. 0.57 cm$^2$) was polished with sandpaper until appearing smooth, rinsed, and incubated in 1% w/w PEI solution for 20 min. Next, the electrode was washed 3 times with water and incubated in a solution containing EDC (50 mM), NHS (50 mM), and PQQ (20 µM) made in HEPES-buffer (10 mM, pH 7.5) for 3 hours. The electrode was then washed and stored in HEPES-buffer (10 mM, pH 7.5). The details of the PQQ immobilization procedure and the modified electrode characterization were reported elsewhere.$^2$

Potential measurements of the PQQ electrode vs. an Ag|AgCl|KCl 3M (Metrohm) reference electrode were performed in Tris-buffer (100 mM, pH 7.4), containing 20 mM CaCl$_2$ in the absence of NADH and in the presence of NADH generated in situ through biocatalytic reactions; note the use of Ca$^{2+}$ cations for promoting NADH oxidation by PQQ$^{[3]}$), Figure S7.

Operation of 4-input-AND concatenated gates. Maltose (Input A, 2 mM for logic value 1), sodium phosphate (Input B, 2 mM for logic value 1), ATP (Input C, 1 mM for logic value 1), NAD$^+$ (Input D, 2 mM for logic value 1), MPh (2 U/mL), HK (2 U/mL), and G6PDH (2 U/mL) were combined in Tris-HCl buffer (100 mM, pH 7.4, containing 20 mM CaCl$_2$) and used to generate NADH in situ. Logic values 0 for the inputs A-D were defined as the absence of the corresponding chemicals. The logic inputs were used in 16 different combinations. The solution was allowed to incubate for 30 min at 30 °C and then reacted with the PQQ-modified electrode.

Operation of 3-input-OR gate followed with AND gate. Glucose (Input A, 2 mM for logic value 1), glucose-6-phosphate (Input B, 1 mM for logic value 1), ethanol (Input C, 85 mM for logic value 1), NAD$^+$ (Input D, 1.5 mM for logic value 1), GDH (1 U/mL), AlcDH (4 U/mL), and G6PDH (50 mU/mL) were combined in Tris-HCl buffer (100 mM, pH 7.4, containing 20 mM CaCl$_2$) and used to generate NADH in situ. Logic values 0 for the inputs A-D were defined as the absence of the corresponding chemicals. The logic inputs were used in 16 different combinations. The solution was allowed to incubate for 30 min at 30 °C and then reacted with the PQQ-modified electrode.

Release of oligonucleotide from the alginate-modified electrode. The oligonucleotide OP1 entrapped in the alginate film on the graphite electrode was released upon reductive dissolution of the alginate matrix when the alginate-modified electrode was electrically connected to the PQQ-modified electrode in the presence of the biocatalytically produced NADH (meaning logic output 1 produced by the enzyme-based logic systems), Figure S3. Minor leakage of the oligonucleotide from the alginate matrix was also observed in the absence of NADH (meaning logic output 0 produced by the enzyme-based logic systems). The oligonucleotide concentration released (leaking) from the alginate matrix was measured by constructing a calibration curve of known concentrations of the same oligonucleotide in mimicked release buffer containing all components of the working system. The calibration curve and the released concentrations were measured by monitoring the fluorescence signal produced by the FITC-label attached to OP1.

Figure S3. Microscope images of the graphite electrode coated with the Fe$^{3+}$-cross-linked alginate thin-film (A) and the same electrode after complete electrochemically stimulated dissolution of the film (B).
**Operation of DNA logic systems.** All DNA computing experiments were conducted in a solution containing 50 mM HEPES, 50 mM MgCl₂, 20 mM KCl, 120 mM NaCl, 0.03% Triton X-100, 1% DMSO at pH 7.4, 200 nM F substrate and 10 nM of all 3iANDa and 3iANDb. OP1 concentration was set by the system as a function of the enzyme logic inputs, 30 °C, 30 min. Figure S4 shows the raw data with the fluorescence spectra obtained with the *in situ* released OP1 (logic value 1) and with the uncontrolled leakage of OP1 (logic value 0). Note that the fluorescence values shown in Figure 4 in the main paper were normalized to 100, while in Figure S4 they have instrumental values before normalization (raw experimental data).

![Fluorescence spectra](image)

**Figure S4.** Fluorescence spectra of the dye-labelled OP1 measured in a solution upon its none-controlled leakage and electrochemically stimulated release. These spectra (and other similar spectra) were used to construct the bar-charts in Figure 4.

**Polyacrylamide gel analysis of 2iAND gate.** The samples containing 200 nM F substrate mimic, 10 nM 3iANDa and 3iANDb, and different input combinations in a buffer containing 50 mM Tris-HCl, pH 7.4; 50 mM MgCl₂; 20 mM KCl, 120 mM NaCl; 0.03% Triton X-100; 1% DMSO, 5 μM FeCl₂, 0.375 mg/mL sodium alginate were incubated at 30°C for 30 min and analyzed by 10% native PAAG. The gel was stained with SYBR Gold and visualized using a UGENEUS Imaging System (Syngene, MD).

In order to confirm the formation of the complex shown in Figure 5B (in the main paper), we employed native polyacrylamide gel electrophoresis, Figure S5. The 3iAND gate was analyzed in the absence of all inputs (lane 1); in the presence of all possible combination of two inputs (lanes 2-4); and in the presence of all 3 inputs (lane 5, last bar in Figure 5B). It can be seen that the low mobility complex indicated by the red arrow is formed only in the presence of all three inputs. The formation of the complex correlates with high fluorescence signal of the gate in the presence of all inputs (Figure 6B, last bar). This complex corresponds to the structure depicted in Figure 5B. Overall, the data of polyacrylamide gel electrophoresis (PAGE) analysis is in accordance with the fluorescent results and supports the hypothesis of the formation of catalytically active complex depicted in Scheme S1 in the presence of all 3 inputs.
Figure S5. Analysis of 3iAND in polyacrylamide gel (PAAG) in the presence of different input combinations. The samples contained oligonucleotides as indicated below each lane. Input combination in the samples corresponded to those for the first bar and the last 4 bars shown in Figure 5B. Lane L contained 25 bp DNA ladder (Promega). The positions of 3iANDa, 3iANDb and the oligonucleotide inputs are indicated. The full complex (198 nucleotides) shown in Scheme S1 is indicated by the red arrow. The complex migrates roughly as a 125-bp DNA marker.


The present detailed procedure starts from the step when the modified electrodes (alginate-modified electrode with the entrapped DNA and PQQ-modified electrode responding to the biocatalytically produced NADH) are ready for the use. The electrode preparation steps are described above.

**Step 1. Assembling the electrochemical cell.**

The electrochemical cell composed of two sub-units (see Figure S6) was used in the experiments. One cell sub-unit was used for the enzyme-biocatalyzed reactions mimicking logic gates and producing NADH as the final output of the enzyme cascades. Another sub-unit was used for the electrochemically stimulated DNA release from the alginate-modified electrode followed by the DNA reaction process. It should be noted that the electrolyte solutions applied to both sub-units were different and they were optimized for different processes performed in the sub-units. The electrolyte solution in the enzyme-subunit was Tris-buffer (100 mM, pH 7.4), containing 20 mM CaCl₂ and the electrolyte solution in the DNA-subunit was 50 mM HEPES-buffer, 50 mM MgCl₂, 20 mM KCl, 120 mM NaCl, 0.03% Triton X-100, 1% DMSO at pH 7.4. These electrolyte solutions also included corresponding enzyme and DNA components as described above. The first sub-unit (used for the enzyme-reactions) included a graphite electrode modified with PQQ catalytic entities. The second sub-unit (used for the DNA release and DNA reactions) included a graphite electrode modified with the Fe³⁺-cross-linked alginate film with the entrapped DNA (OP1). The electrodes were disconnected at the beginning of the experiment, but they were connected electrically with the metallic wire at the later experimental steps (see explanations below). The electrochemical cell sub-units were also connected with a salt bridge providing ionic electrical contacting of the electrolyte solutions in both sub-units. The salt bridge was prepared from a polyethylene tube, 1 mm internal diameter, filled with phosphate buffer, 0.05 M, pH 7, with added Na₂SO₄ 0.1 M.
for the improved conductivity. The ends of the polyethylene tube were closed with ion-permiable membranes made of filter paper.

**Figure S6.** The photo shows the electrochemical system composed of two sub-systems for the enzyme-reactions and for DNA-reactions, respectively, with electrical connections between the sub-systems. Note that this photo shows the electrochemical cells standing on a laboratory bench for better visibility. In the real experiment the cells were immersed into a thermostat for maintaining the temperature of 30 °C.

**Step 2. Performing enzyme reactions in the first cell sub-unit.**

Two different example systems were realized in this cell sub-unit: (a) 4-input-AND concatenated gates and (b) 3-input-OR gate followed with AND gate. The enzyme composition and the corresponding chemical inputs are specified above for both systems in the corresponding paragraphs. The chemical inputs were applied to the cell solution in all possible combinations (16 variants). At this reaction step the PQQ-modified electrode was removed from the cell. The reaction was allowed for 30 min and the cell solution was thermostated at 30 °C. This reaction time was enough to complete all enzyme-biocatalyzed steps and to obtain the final NADH concentration in the solution. The NADH concentration obtained after 30 min reaction was dependent on the combination of the applied input signals according to the logic implemented in the reaction scheme. In other words, the "successfull" input combination resulted in the NADH formation, while the "wrong" input combination preserved NAD⁺ without production of NADH. After the 30 min reaction process, the PQQ-electrode was immersed into the reaction solution and then connected with the conducting wire to the alginate electrode which was included in the second cell sub-unit. It should be noted that the PQQ-modified electrode was not immersed into the reacting solution from the beginning only for the following reasons: (i) We wanted to measure the NADH concentration produced in the reaction volume for all possible input combinations prior to its consumption through the electrochemical reaction. (ii) We wanted to observe the kinetics of the potential formation on the PQQ-modified electrode after the NADH production is completed (without complications from the enzyme-reaction kinetics).
These reasons are only motivated by the research aims, but not mandatory for the whole process performance. In other words, if we do not need to measure the results of the intermediate steps, the PQQ-electrode could be included into the reaction volume from the very beginning.

**Step 3. Measurements of the NADH production by the enzyme biocatalytic cascades.**

An aliquote of the reacting solution was taken from the first cell sub-unit and optical absorbance measurements were performed with a spectrophotometer to derive the NADH concentration, Figure S8. This experimental step is not mandatory and can be omitted. It is not needed for the DNA release triggered by the electrochemical signal.

**Step 4. Measurements of the electrical potential produced on the PQQ-modified electrode (aside step performed separately from the whole process).**

This step was performed in a different electrochemical cell aside from the whole process. The used cell was filled with Tris-buffer (100 mM, pH 7.4), containing 20 mM CaCl$_2$ including all other enzyme/substrate components needed for realization of the enzyme-biocatalyzed cascades (similar to Step 2 described above). The cell included the PQQ-modified electrode and a reference electrode (Ag/AgCl|KCl, 3 M), which were immersed into the reaction solution after 30 min of the enzyme reaction process. The potential produced on the PQQ-electrode was dependent on the absence or presence of NADH and it was measured with a multimeter (Meterman 37XR), Figure S7. It should be noted that this experimental step was performed separately from all other steps and it is not needed for the final result of the DNA release.

**Figure S7.** The photo shows the multimeter measuring the potential produced on the PQQ-modified electrode (vs. Ag/AgCl reference electrode) in the presence (or absence) of NADH produced biocatalytically by the enzyme cascades mimicking logic operations. Note that this photo shows the electrochemical cell standing on a laboratory bench for better visibility. In the real experiment the cell was immersed into a thermostat for maintaining the temperature of 30 °C.

**Step 5. DNA release and following DNA reactions.**
This step started from the moment when both modified electrodes were connected with the electrical wire allowing the current passing from the biocatalytic PQQ-modified electrode (operating in the enzyme sub-unit) to the alginate-modified electrode (operating in the DNA sub-unit). This current resulted in the electrochemical reduction of Fe$^{3+}$ cations in the alginate matrix and thus in the electrochemically stimulated dissolution of the alginate thin-film with the concomitant release of DNA (OP1). The released OP1 then reacted with other DNA components (as described in the paper, see Figure 5) resulting in the formation of fluorescent species. An aliquote of the DNA reacting solution was taken from the cell sub-unit after 60 min of the DNA reaction and analyzed for the fluorescence intensity ($\lambda_{\text{emission}} = 517$ nm with $\lambda_{\text{excitation}} = 485$ nm), Figure S8. It should be noted that the DNA "leakage" corresponded to the situation when the enzyme system did not produced NADH ("wrong" combination of the input signals), while DNA "release" was measured when the NADH was produced ("successful" combination of the input signals) and the PQQ-electrode potential was ca. -60 mV.

**Figure S8.** Measuring NADH absorbance and DNA-fluorescent signal. Note that this photo (center) shows the electrochemical cells standing on a laboratory bench for better visibility. In the real experiment the cells were immersed into a thermostat for maintaining the temperature of 30 °C.

**References**

