ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 3815–3817

www.rsc.org/chemcomm

COMMUNICATION

DNAzyme logic-controlled biofuel cells for self-powered biosensors†

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Received 20th January 2012, Accepted 20th February 2012
DOI: 10.1039/c2cc30464c

The integration of a biosensor employing a DNAzyme logic system within a biofuel cell is presented. The self-powered DNAzyme logic biosensor conforms with INH logic operation and generates power output in accordance with a truth table. The new concept of logic-activated DNAzyme by the input signals has wide-ranging implications in the self-powered diagnostics domain.

Biofuel cells (BFCs) represent a specific variety of novel power sources which employ enzymes or microbes as a bioprocessing contingent to oxidize a biofuel, rather than relying on traditional practices of employing precious metal catalysts for this process.[1] In light of their relatively inexpensive components, ability to process renewable biofuel sources, as well as their potential application as power sources for bionic implants and bioelectronics, BFCs have been widely investigated over the past decade.[1,2] Besides the engineering challenges that target extended operational stability, improved power output, and a higher degree of miniaturization,[1–3] several interesting and attractive studies have recently been reported that aim at the creation of logic-based biosensing systems that harness BFCs for potential self-powered medical diagnostics and therapeutics.[4] By introducing an aptamer-based logic system into a BFC, Dong and colleagues described in 2010 the first self-powered logic aptasensor.[5] Later, they introduced the enzyme logic concept into a BFC towards the realization of the first self-powered logic-based enzymatic biosensor.[6] In 2011, Angenent and co-workers further reported the first instance of self-powered microbial biosensors based on BFCs.[7] Recently, Wang's group reported the first self-powered "Sense-Act-Treat" system that is based on a BFC and controlled by Boolean logic, which offers simultaneous biocomputing diagnostic operation and controlled drug-release functionality without the need for external power sources.[4c] Compared with traditional biosensors, one of the most significant advantages of logic-based biosensing systems integrated within BFCs resides in their ability to correlate the relationship between multiple target analytes in complex samples according to the principles of Boolean logic without the need for external power sources or control electronics.[8]

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2cc30464c
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In this communication, we describe the first example of the ability to control the power output generated by a BFC using DNAzyme-based biochemical signals processed according to Boolean logic operations. DNAzymes, selected by a systematic evolution of ligands by an exponential enrichment process (SELEX), are biocatalytic nucleic acids with a promising capacity to selectively identify charged organic and inorganic species at ultra-trace levels.[9] Because of their unique characteristics, DNAzymes exhibit many intrinsic advantages over conventional enzymes, including high chemical stability, cost-effective reproducible synthesis, and facile chemical modification.[9,10] Thus, their unique properties have made these catalytic nucleic acid systems attractive in diverse areas for versatile applications.[9,10] While DNAzymes have been widely used for biosensing, nanomachines, logic gate applications,[9b,c,10,11] as well as for the cathode component within BFCs,[8] the present work represents, to the best of our knowledge, the first example of a DNAzyme logic-controlled BFC for implementation as a self-powered biosensor.

Harnessing a built-in INH logic gate, the DNAzyme-controlled BFC enables the construction of a self-powered DNAzyme logic biosensor that can determine the presence of one specific target in the absence of another related target in a single test. The INH logic gate integrates the functional properties of AND and NOT gates, where the output signal is activated by a specific combination of the inputs according to a truth table.[12] The development of a logically-controlled BFC by different DNAzyme-based input signals holds considerable promise for the construction of potential "smart" self-powered biosensors or related bioelectronic diagnostic devices. As illustrated in Fig. 1, the DNAzyme–BFC system is comprised of a hairpin DNA-modified cathode and a glucose dehydrogenase (GDH)-modified anode. At the cathode, a horseradish peroxidase (HRP)-mimicking DNAzyme sequence and an adenosine monophosphate (AMP)-binding aptamer sequence are both blocked in the stem region of a hairpin DNA sequence (Fig. 1 and Fig. S1 in ESI†). Upon exposure of the cathode to AMP in the presence of hemin, the AMP-binding aptamer–AMP complex forms, resulting in the opening of the hairpin stem and hence the self-assembly of the hemin/G-quadruplex HRP-mimicking DNAzyme which electrocatalyzes the reduction of H_2O_2 (Fig. 1 and Fig. S1 in ESI†). Control experiments reveal that only the treatment of both hemin and AMP on the cathode results in notably increased H_2O_2 reduction currents compared with the absence of hemin or AMP (Fig. S2 and S3 in ESI†). This entails that the HRP-mimicking DNAzyme formation based

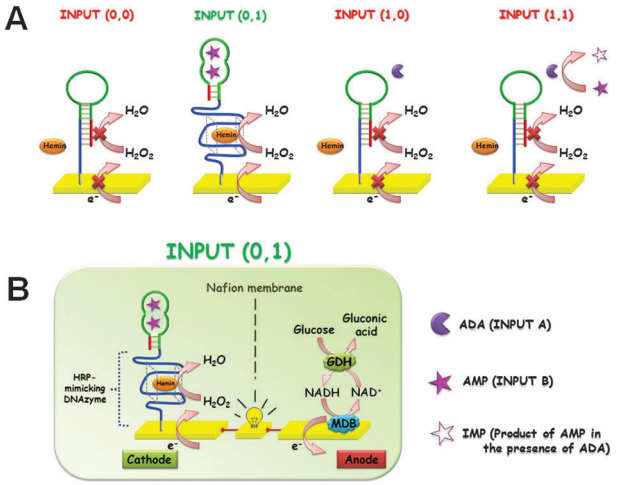
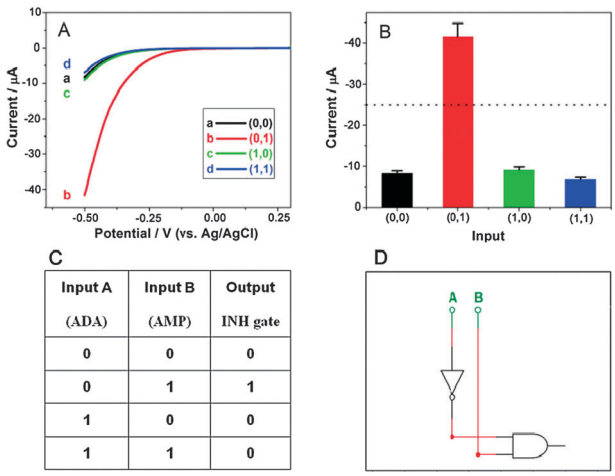


Fig. 1 (A) Schematic illustration of the logically-controlled cathode by different DNAzyme-based input signals. (B) Schematic illustration of the BFC logically controlled by DNAzyme-based input (0,1). Input A, ADA; Input B, AMP.



Input A (ADA)	Input B (AMP)	Output INH gate
0	0	0
0	1	1
1	0	0
1	1	0

Fig. 2 (A) Linear scan voltammograms at the cathode for 1 mM H_2O_2 upon different input signals (0,0) (a), (0,1) (b), (1,0) (c) and (1,1) (d). Scan rate: 2 mV s^{-1}. (B) Bar diagrams, illustrating the current response at −0.5 V generated by all permutations of the input signals, derived from Fig. 3(A). The dotted line indicates the threshold (−25 μA). (C) Truth table for the INH logic gate. (D) Circuit for the INH logic gate. Input A, 10 U mL^{-1} ADA; Input B, 50 μM AMP. Cathode electrolyte: N_2-saturated 10 mM pH 7.2 HEPES buffer containing 50 mM KCl, 100 mM NaCl, and 1 μM hemin.

on hemin and AMP on the electrode interface can be used as the cathode of a BFC operating in an N_2-saturated 10 mM pH 7.2 HEPES buffer (containing 50 mM KCl, 100 mM NaCl, and 1 μM hemin) with H_2O_2 (1 mM) as the oxidizer. Using an anode modified with a GDH (E.C. 1.1.1.47, 256 U mg^{-1})–carbon nanotubes (CNT)–Meldola's blue (MDB) composite, glucose biofuel (24 mM) was oxidized in the presence of NAD^+ in an N_2-saturated 0.1 M pH 7.2 phosphate buffer solution (PBS) (Fig. S6 in ESI†). MDB was employed as the mediator to aid in the oxidation of glucose by GDH. The cathode and anode were separated with a Nafion membrane (Nafion® 117, thickness 0.007 in.). The design of the BFC, Fig. 1, clearly demonstrates the components of a self-powered intelligent logic DNAzyme biosensor.

In order to demonstrate proper DNAzyme logic operation, adenosine deaminase (ADA) (which can transform the AMP substrate into inosine monophosphate (IMP)) and AMP were applied as the model inputs (inputs A and B, respectively). The absence of ADA or AMP was considered as logic input 0, while their optimized concentrations at 10 U mL^{-1} for ADA and 50 μM for AMP were defined as logic input 1. The input signals were applied in all four combinations ((0,0), (0,1), (1,0), and (1,1), where the first bit corresponds to signal A and the second bit to signal B). When ADA and AMP were absent (input (0,0) in Fig. 2A), the current for the H_2O_2 reduction at the cathode was −8.62 μA (at −0.5 V *vs.* Ag/AgCl). Following the addition of AMP, significantly higher current was obtained (−40.60 μA) due to the formation of the hemin/G-quadruplex HRP-mimicking DNAzyme on the cathode (input (0,1) in Fig. 1A and Fig. 2A and B). When only ADA was present (input (1,0) in Fig. 1A and Fig. 2A and B), the DNAzyme did not form on the cathode, thereby leading to output 0. When ADA and AMP were both present (input (1,1) in Fig. 1A, Fig. 2A and B and Fig. S4 and S5 in ESI†), ADA transformed the AMP in base solution (or the generated AMP binding aptamer–AMP complex on the cathode transformed into IMP), which resulted in an output of 0. Therefore, the features of the system correspond to the equivalent circuitry of an INH logic gate performing the Boolean logic operation of $A'B$ (Fig. 2C and D and Fig. S4 and S5 in ESI†). Specific patterns of the ADA and AMP BFC inputs thus generate an output in compliance with a truth table for an INH gate.

The logically-controlled H_2O_2-reducing cathode was subsequently coupled with a glucose-oxidizing anode (Fig. S6 in ESI†) to yield a complete BFC. The application of the (0,0) input signal did not alter the power output of the BFC (input (0,0) in Fig. 3A and B and Fig. S7, ESI†). When the (0,1) input signal was applied, the hemin/G-quadruplex HRP-mimicking DNAzyme formed at the cathode interface; accordingly, the power output increased, thus resulting in the ON state of the BFC (input (0,1) in Fig. 3A and B and Fig. S7, ESI†). When the (1,0) and (1,1) input signals were applied, the power output of the BFC did not change appreciably; accordingly, the BFC remained in the OFF state (inputs (1,0) and (1,1) in Fig. 3A and B and Fig. S7, ESI†).

The INH function can be interpreted as a particular integr ation of AND and NOT logic functions, where the output signal is activated by a specific combination of the active inputs (Fig. 3C and D).[12] If only AMP is present in the sample (input (0,1)), a significantly elevated power output is observed (output 1) and the gate switches ON (input (0,1) in Fig. 3). If AMP is not present or AMP and ADA are both in the sample (inputs (0,0), (1,0) and (1,1)), the power output is "disabled" (output 0); consequently, the INH gate switches the BFC to the OFF state (inputs (0,0), (1,0) and (1,1) in Fig. 3). Therefore, the INH logic gate presented here queries only the presence of a specific target in the absence of another related input in a single test. Additionally, the present DNAzyme INH logic-based device is able to operate in the absence of an external power source, hence underscoring the inherent self-powered characteristics of this bioelectronic device. Testing the stability of the new device yielded decay of the power output

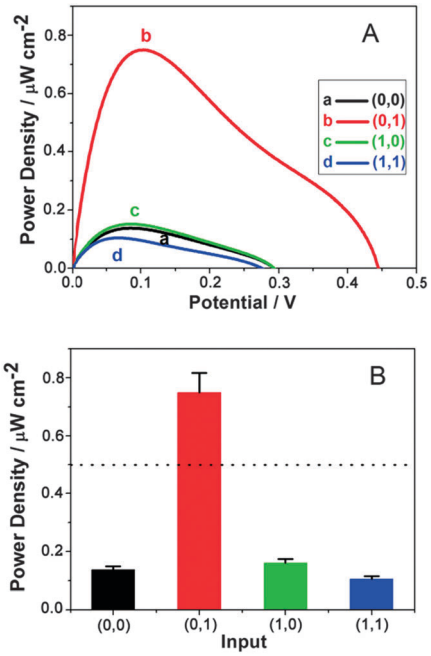


Fig. 3 (A) Dependence of the power density on the BFC voltage. Anode biofuel, 24 mM glucose; cathode oxidizer, 1 mM H_2O_2 in connection to different input signals (0,0) (a), (0,1) (b), (1,0) (c) and (1,1) (d). (B) Bar diagrams, illustrating the maximum power density for the different combinations of the input signals, derived from (A). The dotted line is established at the threshold (0.5 μW cm^{-2}). Anode electrolyte: N_2-saturated 0.1 M pH 7.2 PBS containing 20 mM NAD^+. Cathode electrolyte: N_2-saturated 10 mM pH 7.2 HEPES buffer containing 50 mM KCl, 100 mM NaCl, and 1 μM hemin.

(with up to 5% and 30% after 20 and 40 min, respectively (data not shown)) by the response of the (0,1) operation. However, considering that each biosensor was designed to be used only once and each measurement can be readily finished within 5 min, such a biosensor still accurately performed the INH function (Fig. 3). No stability data are available for other newly developed self-powered biocomputing logic biosensors based on BFCs.[4c,5–7]

In conclusion, we have introduced, for the first time, a DNAzyme logic system into a BFC in order to provide for novel self-powered DNAzyme logic biosensing applications. This *proof-of-concept* study employs a pattern of ADA and AMP as inputs to a BFC, which generates an output in compliance with a truth table for an INH gate, based on modulating the HRP-like activity of DNAzyme. The concept also demonstrates the feasibility of self-powered medical diagnostics using a biologically-inspired logic gate-based design. If pathologically-relevant targets are applied to the DNAzyme logic-controlled BFC, self-powered diagnostics may be realized, which would enable the direct screening of various medical conditions. In view of the tremendous interest in DNAzyme-based bioanalytical systems,[9,10] the eventual integration of DNAzymes with self-powered biocomputing devices holds considerable potential for diverse applications.

It should also be noted that DNAzymes exhibit many advantages over conventional enzymes,[9b,c] which could make DNAzymes versatile for various "smart" logic sensing applications. While this initial concept of logic-based activation of DNAzyme activity has been illustrated using the model AMP and ADA compounds, it can be expanded to the determination of metal ions (*e.g.*, Ag^+, K^+, Hg^{2+}, Pb^{2+} or Cu^{2+}), amplified detection of small molecules such as adenosine, cocaine, or AMP, and the identification of proteins such as lysozyme or thrombin.[9a,13]

This work was supported by the Office of Naval Research (ONR Award #N00014-08-1-1202) and the National Science Foundation (Awards CBET-1066531 and CHE-1057562).

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