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Colorimetric logic gates based on aptamer-crosslinked hydrogels†

Bin-Cheng Yin^a, Bang-Ce Ye^a, Hui Wang^b, Zhi Zhu^b, and Weihong Tan^b

^aLab of Biosystem and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science & Technology, Shanghai 200237, P.R. China. bcye@ecust.edu.cn; Fax: 0086-21-64252094; Tel: 0086-21-64252094

^bCenter for Research at the Bio/Nano Interface, Department of Chemistry and Physiology and Shands Cancer Center, University of Florida, Gainesville, FL 32611-7200, USA. tan@chem.ufl.edu

Abstract

We have developed a novel molecular logic gate system based on the incorporation of aptamer-crosslinked hydrogels. Modified gold nanoparticles are used as the output signal, which is visible to the naked eye. This system is designed for AND and OR operations using two chemicals as stimulus inputs.

It is well known that semiconductor logic gates form the basis of conventional silicon computer microprocessors employing electronic input and output signals.¹ In recent years, molecular logic gate systems have been extensively studied, with the goal of the controlled assembly of biomolecules that can give intelligent responses to the external stimuli.² Toward this goal, many diverse supramolecular systems based on smart materials³ have been rationally designed and constructed to provide macroscopic outputs in response to controlled inputs.

In particular, a supramolecular hydrogel has attracted significant research interest based on its macroscopic gel–sol behavior in response to different stimuli.⁴ To date, various hydrogels have been reported in response to a broad spectrum of stimuli, including temperature,⁵ pH,⁶ ionic strength,⁷ light,⁸ and electric fields.⁹ These hydrogels can directly cause mechanical changes based on gel swelling and shrinking, or property changes in optical transmission, refractive index, and resonance frequency.

Recently, we have developed a photoresponsive DNA hydrogel that can be utilized for precisely controllable encapsulation and release of multiple loads.¹⁰ We also have developed a general design for a colorimetric visual detection platform based on the stimulus-responsive gel–sol transition of a DNA hydrogel.¹¹ Interestingly, the concept of DNA assembly has been expanded to realize an unconstrained three-dimensional DNA

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Correspondence to: Bang-Ce Ye; Weihong Tan.

hydrogel.¹² Acrydite-modified DNAs incorporated with polymer chains have been used to build functional hydrogels, which are biocompatible and thus maintain the native structure of the DNA. Herein, to explore new dimensions in molecular logic gates, we have built a system of colorimetric logic gates (AND and OR) responsive to the control of chemical stimuli. This system is based on the concept of combining aptamer-crosslinked hydrogels with smart materials.

Aptamers are single-stranded oligonucleotides, which can fold into unique tertiary structures for specific molecular recognition.¹³ They are selected by SELEX (systematic evolution of ligands by exponential enrichment)¹⁴ or various modified formats of SELEX.¹⁵ Like antibodies, aptamers specifically bind to a wide variety of targets ranging from macromolecules to small compounds. But aptamers also exhibit a number of advantages over antibodies, such as good thermal stability, simple synthesis and easy labeling.¹⁶ Highly target-responsive hydrogels can be achieved by incorporating aptamers into the network.

To demonstrate the feasibility of this concept, we employed ATP/cocaine-responsive hydrogels as a model system. Fig. 1 shows the construction of the AND logic gate. The AND logic gate is represented by the situation where the output is true only if both inputs are true. Two pieces of acrydite-modified DNA, S1 (5'-acrydite-AAAACCTCATCTGTGAAAGAACCTGGGGGAGTATTGCGGAGGAAGGT-3') and S2 (5'-acrydite-AAACCCAGGTTCTTCTAGAGGGAGAC-3'), are copolymerized with linear polyacrylamide polymers to form polymer strands P-S1 and P-S2 in a transparent liquid form, respectively. S1 is designed to contain an ATP aptamer fragment.¹⁷ A crosslinker L1 (5'-GGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCCCTCTACTCACAGATGAGT-3'), containing a cocaine aptamer fragment,¹⁸ is designed to hybridize with S1 and S2, respectively. When P-S1 and P-S2 are mixed with L1, the polymers transform into a gel as the hybridization proceeds. The hybridization format is designed in such a way that these three DNA strands form a Y-shaped structure, with each strand containing two half-complementary domains for the other two strands. In other words, each DNA strand can bind to any other strand through base pairing to produce the Y-shaped DNA (see Table S1 in the ESI† for detailed sequence design). The hybridization process leads to an extended three-dimensional network in solution, and yields a highly viscous DNA hydrogel.¹⁹

To help visualize the sol/gel transition, we employed BSA-modified gold nanoparticles (BSA-GNPs) as indicator reagents because of their unique optical properties and chemical stability. GNPs give a remarkably large extinction coefficient at the visible wavelength of 522 nm, which makes them a sensitive indicator reagent for visual detection, and BSA has been found to stabilize the GNPs against aggregation under high ionic strength conditions. In our experiment, the BSA-GNPs are thoroughly mixed with P-S1 and P-S2 before hydrogel formation. Then crosslinker L1 is introduced, and a homogeneous red-colored hydrogel is formed with trapped BSA-GNPs inside. After washing buffer solution is added to remove surface-bound BSA-GNPs, the resulting BSA-GNP-entrapped hydrogel is very stable with no BSA-GNP release observed, even after several days' storage without stirring.

Upon introduction of ATP, the ATP aptamer fragment in P-S1 switches its structure and binds two ATP molecules. Because of the decrease in the number of complementary base pairs from eleven to four, P-S1 dissociates from P-S2. However, the network of the hydrogel remains intact, since crosslinker L1 still keeps P-S1 and P-S2 together. Likewise, upon introduction of cocaine, the cocaine aptamer fragment in crosslinker L1 switches its structure, binds one cocaine molecule, and dissociates from P-S2. The presence of cocaine alone does not result in the disruption of the hydrogel due to the linkage of P-S1 and P-S2. However, treatment of the hydrogel with both cocaine and ATP leads to dissolution of the DNA hydrogel and release of BSA-GNPs to the upper buffer solution layer. As a result, the buffer solution turns from colorless to red, a color change that can be easily detected with the naked eye.

These phenomena are shown in Fig. 2A. It can be clearly seen that no color change was observed when the system was subjected to one stimulus input, ATP (1,0), cocaine (0,1) or the absence of any input (0,0). On the other hand, when the system was subjected to both stimulus inputs, a color change of the solution was obtained corresponding to a “true” output (1,1).

The UV-vis spectrum of the results of the AND gate is shown in Fig. 2B. Upon dissolution of the aptamer-crosslinked hydrogel, a typical extinction of the 522 nm plasmon peak was recorded, which results from the release of BSA-GNPs.

To evaluate the specificity of this system, analogues of cocaine and ATP were investigated. Two cocaine analogues, benzoylecgonine (BE) and ecgonine methyl ester (EME), are reported to have no affinity for the cocaine aptamer.¹⁸ Therefore, they were employed as negative controls. Four ATP analogues of guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP) and thymidine triphosphate (TTP) were employed to examine the selectivity of the sensor to ATP. As shown in Fig. 2C and D, no tested analogues caused hydrogel dissolution or color change in a manner similar to ATP or cocaine. These results demonstrate the successful construction of the AND logic gate.

Fig. 3 shows the construction of the OR logic gate. The OR logic gate is represented by the situation where the output of an OR logic gate is true if either input is true. Similar to the AND gate, two pieces of acrydite-modified DNA, S3 (5'-acrydite-AAACCCAGGTTCTCT-3') and S4 (5'-acrydite-AAATGAGAGGGAGAC-3'), are grafted onto linear polyacrylamide polymers to form polymer strands P-S3 and P-S4, respectively. In this design, a crosslinker L2 (5'-GGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCCCTCTCAAGAGAACCTGGGGAGTATTGCGGAGGAAGGT-3') is designed to contain both a cocaine aptamer fragment at the 5'-end and an ATP aptamer fragment at the 3'-end. The addition of L2 initiates the hybridization of P-S3 and P-S4, with the partial aptamer sequences in L2, respectively, thus crosslinking the linear polyacrylamide polymers to form the hydrogel. In the presence of either ATP or cocaine, the number of base pairs between L2 and P-S3 or P-S4 is decreased to four, a number which is not strong enough to maintain the linkage. Therefore the hydrogel dissolves to release the BSA-GNPs as a result of the competitive target-aptamer binding, giving rise to the OR logic gate. Thus, a color change was obtained

when the system was subjected to one stimulus input, ATP (1,0), cocaine (0,1), or two stimulus inputs (1,1). The photographs are shown in Fig. 4A with the truth table. We also investigated the system by UV/Vis spectroscopy (Fig. 4B).

To evaluate the specificity of this system, the analogues of ATP (GTP, CTP, UTP and TTP) and cocaine (BE, EME) were investigated. As shown in Fig. 4C and D, the logic gates demonstrate good selectivities for ATP and cocaine inputs compared with the studied analogues. Taking all these results together, the use of aptamer-crosslinked hydrogels for the fabrication of colorimetric OR logic gates is validated.

In summary, we have engineered hydrogel-based supramolecular logic gates displaying colorimetric visual AND and OR functions with cocaine and ATP as the input stimuli. The specific aptamer–target recognition enables such DNA hydrogels to undergo a macroscopic gel–sol transition in response to the applied logic stimuli. Taking advantage of the hydrogel’s physicochemical behaviour, target-triggered release of trapped cargos, such as the BSA-GNPs used here, is directly visualized, demonstrating the potential of the aptamer-switchable hydrogel as a controlled release system with possible applications in biosensors, nanomechanical devices and drug delivery devices. Moving forward, we envision that this functional hydrogel can be programmed to respond to a wide range of targets by simply engineering functional nucleic acids, thus providing a universal platform for polymeric logic gates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Biography

Bin-Cheng Yin

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Fig. 1. AND logic gate system based on aptamer-crosslinked hydrogels and activated by the presence of cocaine and ATP.

Fig. 2. AND logic gate characterized by colorimetric and UV/Vis detection. (A) Photograph of aptamer-crosslinked hydrogels visualized with trapped BSA-GNPs before and after addition of cocaine and ATP. (B) UV-vis spectrum of the results of the AND logic gate. (C) Photograph of selectivity of the system to cocaine. (D) Photograph of selectivity of the system to ATP. The input stimulus concentration was 1 mM.

Fig. 3.
OR logic gate system based on aptamer-crosslinked hydrogels and activated by different chemical stimuli of cocaine and ATP.

Fig. 4.

OR logic gate characterized by colorimetric and UV/Vis detection. (A) Photograph of aptamer-crosslinked hydrogels visualized with trapped GNPs before and after addition of cocaine and ATP. (B) UV-vis spectrum of the results of the OR logic gate. (C) Photograph of selectivity of the system to cocaine. (D) Photograph of selectivity of the system to ATP. The input stimulus concentration was 1 mM.