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Enzymatic logic calculation systems based on solid-state electrochemiluminescence and molecularly imprinted polymer film electrodes

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ABSTRACT

The molecularly imprinted polymer (MIP) films were electropolymerized on the surface of Au electrodes with luminol and pyrrole (PY) as the two monomers and ampicillin (AM) as the template molecule. The electrochemiluminescence (ECL) intensity peak of polyluminol (PL) of the AM-free MIP films at 0.7 V vs Ag/AgCl could be greatly enhanced by AM rebinding. In addition, the ECL signals of the MIP films could also be enhanced by the addition of glucose oxidase (GOD)/glucose and/or ferrocenedicarboxylic acid ($Fc(COOH)_2$) in the testing solution. Moreover, $Fc(COOH)_2$ exhibited cyclic voltammetric (CV) response at the AM-free MIP film electrodes. Based on these results, a binary 3-input/6-output biomolecular logic gate system was established with AM, GOD and $Fc(COOH)_2$ as inputs and the ECL responses at different levels and CV signal as outputs. Some functional non-Boolean logic devices such as an encoder, a decoder and a demultiplexer were also constructed on the same platform. Particularly, on the basis of the same system, a ternary AND logic gate was established. The present work combined MIP film electrodes, the solid-state ECL, and the enzymatic reaction together, and various types of biomolecular logic circuits and devices were developed, which opened a novel avenue to construct more complicated bio-logic gate systems.

1. Introduction

Molecular logic gates, similar to their electronic counterparts based on silicon-based integrated circuits, can perform various computations but at the molecular level (Andreasson and Pischel, 2015; de Silva and Uchiyama, 2007; de Silva et al., 1993; Stojanovic et al., 2002; Xia et al., 2010). Biomolecular logic gates are a type of molecular logic gates that employ biomaterials or biomolecules such as nucleic acids (DNA), enzymes or proteins to construct the basic molecular devices (Katz, 2015; Willner et al., 2008). In recent years, biomolecular logic gates have attracted increasing attentions from researchers due to their high application potential in different fields including diagnostics, sensing and molecular recognition (Gui et al., 2014; Ling et al., 2015; Park et al., 2010).

One great challenge in developing biomolecular logic gates is to increase the complexity of the system, so that more complicated calculations can be accomplished. One direction in this regard is to increase the number of input and/or output of the logic gates. Early bio-logic gate system had only one or two inputs/outputs (Manesh et al., 2009; Privman et al., 2009). Afterwards, biomolecular logic devices with multiple inputs/outputs were constructed (Erbas-Cakmak et al., 2013; Guz et al., 2014; He et al., 2014; Liu et al., 2015a, 2015b; Shi et al., 2015). The second direction is to develop non-Boolean logic devices. Compared with the traditional Boolean logic gates, the non-Boolean logic devices such as encoder/decoder, multiplexer/demultiplexer and keypad lock can accomplish some special and specific tasks (Gao et al., 2017). The third direction in increasing the complexity is to develop multi-valued logic gates (Ran et al., 2014). Usually, the logic gate system is binary, i.e. it has only two states in its input/output: on and off, or true and false. The disadvantage of binary logic gates is their uncertainty and imprecision especially when more complicated information needs to be processed. Multi-valued logic gate system can make up the imperfection. It involves switches between more than two states, and the increase of the number of states in input/output brings about higher data storage densities and more powerful information processing capability (He et al., 2015). However, only a few studies

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have been reported on the multi-valued logic gates based on biomolecular system until now (He et al., 2015; Pu et al., 2011; Ran et al., 2014).

Electrochemiluminescence (ECL), also referred to as electrogenerated chemiluminescence, is chemiluminescence triggered by electrochemical reactions (Hu and Xu, 2010), which demonstrates some unique advantages such as rapid response, low cost, high sensitivity, and simplified setup (Chen et al., 2014). Usually, cyclic voltammetry (CV) and ECL signals can be measured and obtained simultaneously by ECL instruments. Up to now, however, only a few investigations have been reported by our group on biomolecular logic gates with simultaneous CV and ECL responses as two types of outputs (Lian et al. 2015; Liu at al. 2015b).

The molecular imprinting technology is an approach that uses synthetic molecular imprinted polymer (MIP) with specific binding sites to recognize the target molecule (Chen et al., 2011). With the intrinsic advantages such as high selectivity, good mechanical strength, low cost, and easy preparation, MIP has been successfully applied as the recognition element in quantitative analysis (Erdossy et al., 2017; Kim et al., 2017; Moon et al., 2017; Chantada-Vazquez et al., 2016; Tan-Phat et al., 2013; Ton et al., 2015). Recently, MIP electrochemical and MIP-ECL sensors for the detection of various substances have been developed, which combine the excellent selectivity of MIP and the high detection sensitivity of voltammetry and ECL (Li et al., 2012; Wu et al., 2012; Xue et al., 2014). However, until now, very few reports have been made on the logic gate system on the basis of combination of MIP and ECL (Lian et al., 2015).

In the present work, a series of binary biomacromolecular logic gates and devices, and a ternary logic gate were developed based on the MIP films at electrodes and the solid-state ECL. The solid-state ECL, by immobilizing the ECL reagent on the electrode surface, usually demonstrates advantages over the solution-state ECL in its better signal-to-noise ratio, less consumption of ECL reagent, and simplified experimental design (Fu et al., 2016; Wang et al., 2014). Herein, the ECL reagent luminol and functional monomer pyrrole (PY) were electropolymerized into copolymer MIP films at the surface of electrodes with ampicillin (AM) as the template, designated as AM-PPY/PL MIP (PPY = polypyrrole and PL = polyluminol). AM is a type of β-lactam antibiotics, and is widely used for the treatment of infections in humans and animals (S. Wei et al., 2014). Under aerobic and basic conditions, β-lactam antibiotics such as AM can produce superoxide anion free radical ($O_2^{-\bullet}$) (Kubo et al., 1999), which was used in this work to oxidize PL and enhance its ECL signal. Thus, the ECL response of AM-PPY/PL MIP film system was employed to recognize and detect AM in its rebinding solution (Scheme 1A). On the other hand, the addition of glucose oxidase (GOD)/glucose and/or ferrocenedicarboxylic acid (Fc(COOH)$_2$) in the testing solution could improve the ECL intensity of the system. In addition, Fc(COOH)$_2$ also functioned as the electroactive probe and exhibited CV signal at the AM-free MIP film electrodes. Thus, with AM, GOD and Fc(COOH)$_2$ as inputs, and the corresponding CV and ECL responses as outputs, a binary 3-input/6-output logic gate was established. In addition, some non-Boolean logic devices, including an encoder, a decoder and a demultiplexer, were also constructed based on the same MIP system with elaborate designs. Especially, a ternary AND logic gate was successfully developed on the same platform. To the best of our knowledge, this is the first report on biomolecular multi-valued logic gate based on MIP and ECL. The present system combined MIP and solid-state ECL with the enzymatic reaction, demonstrating great potential in biocomputing. This new idea had generality to some extent, and might become a foundation for the development of some novel and more complicated biomolecular computing devices.

2. Experimental section

2.1. Preparation of MIP film electrodes

MIP films were fabricated on the surface of Au electrode by electropolymerization. First, the Au electrodes were sequentially polished with 1.0, 0.5 and 0.05 μm γ-alumina on chamois leathers to obtain a mirror-like surface, followed by ultrasonication in ethanol and water for 5 min, respectively. After optimization, the electropolymerization of AM-PPY/PL MIP films was performed by 5 cycles of CV scans at Au electrodes between 0 and 1.0 V at 50 mV s^{-1} in the solution containing 60 mM AM template, 20 mM PY and 5 mM luminol monomers in 0.1 M phosphate buffer solutions at pH 6.0. AM-free MIP films were prepared by placing the MIP film electrodes in 20 mL methanol/acetic acid (9:1, v/v) solutions for 20 min with magnetic stirring so that the AM molecules previously entrapped in the MIP films could be removed. The AM-free MIP film electrodes were then rinsed with water and dried at room temperature. The AM-rebinding MIP film electrodes were formed by incubation of the AM-free MIP film electrodes in 0.1 M phosphate buffer solutions at pH 7.0 containing different concentrations of AM for 15 min, followed by water rinsing and room temperature drying.

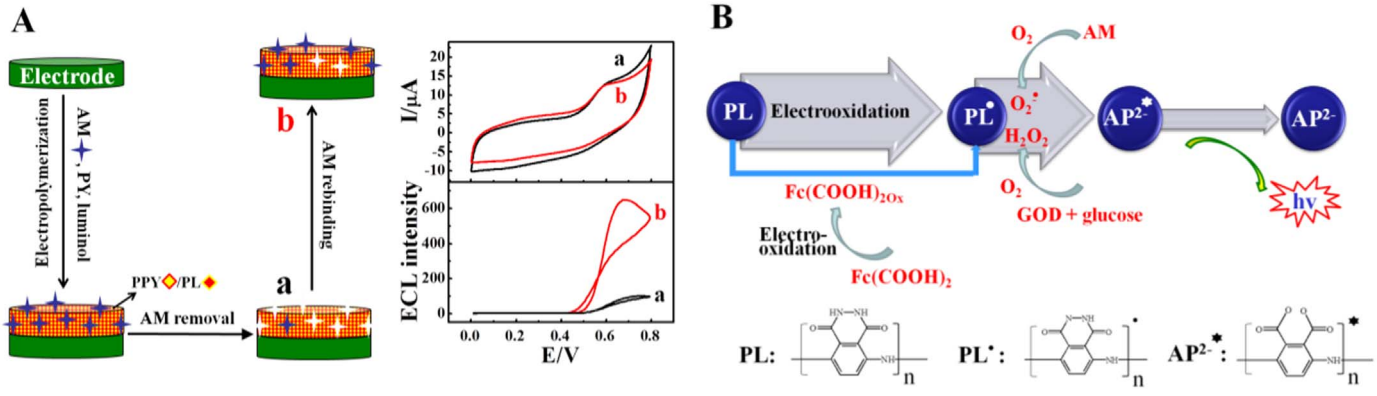
The PL, AM-PL and non-molecularly imprinted polymer (NIP) film electrodes were prepared with the similar method.

The reagents and apparatus used in this work, and the characterization of AM-PPY/PL MIP films, were described in details in Supplementary information.

3. Results and discussion

3.1. Molecular recognition of AM by MIP films

The simultaneous CV and ECL experiments were performed to confirm the molecular recognition function of the MIP films toward AM (Fig. 1). In pH 8.0 blank buffers, a CV oxidation peak at approximate



Scheme 1. Schematic representation of (A) the fabrication process of AM-PPY/PL MIP film electrodes and (B) the possible mechanism of ECL signals controlled by AM, GOD/glucose and Fc(COOH)$_2$.