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Label-free monitoring of the thrombin–aptamer recognition reaction using an array of nanochannels coupled with electrochemical detection

Xiao-Ping Zhao[a,b], Jing Cao[a], Xing-Guo Nie[a], Shan-Shan Wang[a], Chen Wang[a,*], Xing-Hua Xia[b]

[a] Key Laboratory of Biomedical Functional Materials, School of Science, China Pharmaceutical University, Nanjing 211198, China
[b] State Key Laboratory of Analytical Chemistry for Life Science, Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

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ABSTRACT

A sensitive and label-free method of monitoring the thrombin–aptamer recognition reaction has been developed using an array of nanochannels coupled with an electrochemical detection technique. Due to the highly amplified ion current produced by an array of nanochannels compared to a single nanochannel/pore, a significant increase in detection sensitivity has been achieved.

1. Introduction

Molecular recognition plays an important role in biological systems, and is involved in virtually every process within organisms. Monitoring of biomolecular recognition reactions is of vital significance in exploring the functions of biomolecules and their reaction mechanisms and kinetics in living systems. So far, a variety of methods have been developed for assay of biomolecular recognition which involve aqueous solutions [1], organic solutions [2], solid interfaces [3], and microfluidic devices [4–7]. For example, Majumdar et al. demonstrated a label-free antibody–antigen binding assay using two-dimensional microcantilever arrays. By optically measuring the nanoscale motion of two-dimensional arrays of microcantilever beams, protein–protein recognition could be monitored in real-time [3]. These methods are of great significance in advancing the progress of biomolecular recognition studies. However, they also usually require considerable quantities of the biomolecule, which can be difficult to obtain if the target molecule is rare and supply limited. It is therefore desirable to develop simple, sensitive, and miniaturized analytical approaches for molecular recognition studies.

In an alternative approach, nanopores/channels have been widely used in the construction of biosensors and chemical analytical devices in recent years [8–12]. Among their particular attributes, nanopores/channels possess the exquisite ability of revealing a change in molecular volume through a measurable ionic current, which offers great opportunities for the study of biomolecular recognition reactions [13–15]. However, single nanochannel/nanopore-based devices usually have ionic currents with very small amplitudes (usually at the level of the pico ampere). To achieve a satisfactory result, expensive facilities such as patch-clamp equipment or other devices must be used to amplify the change in ionic current [16,17]. In contrast, a porous anodic alumina (PAA) membrane has a high density of nanochannels forming an array, and can deliver an ionic current change several orders of magnitude larger than a single nanopore [18–27]. For example, Merkoçi's group carried out a series of highly sensitive bioanalyses using PAA, including immunoassay, thrombin detection and DNA hybridization [24–27]. In our previous work, amyloid β aggregation kinetics was successfully monitored in real-time using a PAA-based nanofluidic device [20]. In this work a strategy for a label-free biomolecular recognition assay using PAA is proposed. Using the change in the ion current before and after the addition of the target molecule, thrombin–aptamer recognition kinetics can be monitored in real-time. This provides a novel and simple method for label-free monitoring of biomolecular recognition reactions.

2. Experimental section

2.1. Modification of the PAA membrane with the thrombin aptamer (TBA)

The prepared PAA was hydroxylated in boiled hydrogen peroxide (30% H_2O_2) at 98–100 °C for 0.5 h, then immersed in 10% of 3-aminopropyltrimethoxy-silane (APTMS) for 6 h and heated at 120 °C for 2 h to crosslink the silane layer. Next the APTMS-grafted PAA membrane was treated with a mixed solution (1 μM TBA, 0.9 mg/ml EDC and 0.8 mg/ml NHS) for 6 h [23]. Each modification step was followed by rinsing with deionized water. This resulted in a functionalized PAA membrane with the aptamer immobilized on the inner walls

* Corresponding author.
E-mail address: wangchen@cpu.edu.cn (C. Wang).

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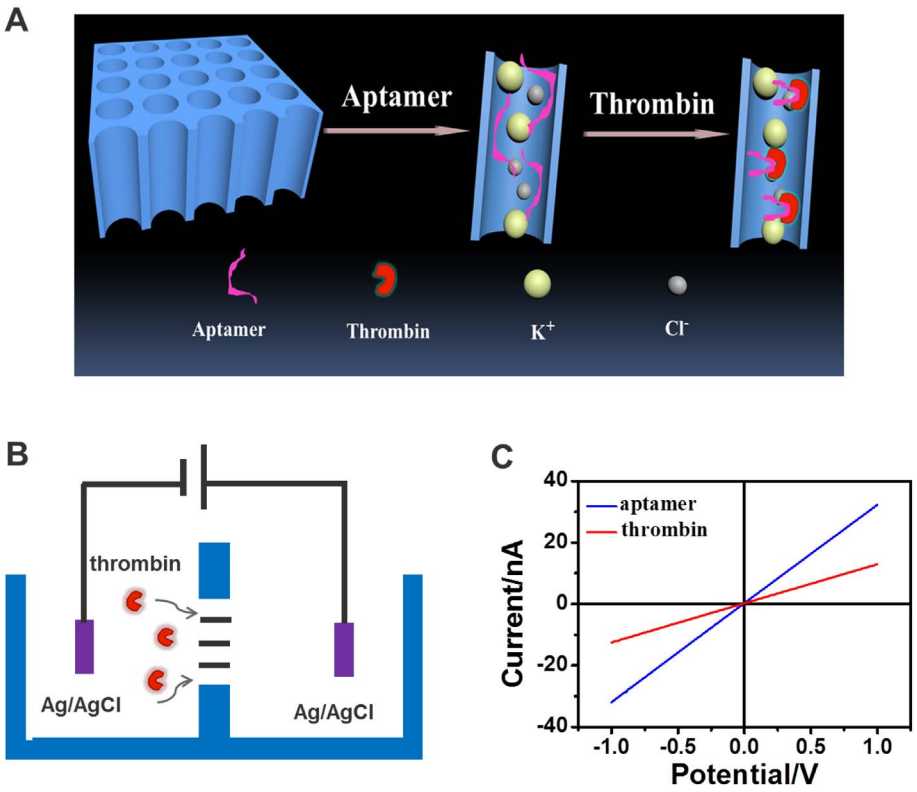

Fig. 1. Label-free assay of thrombin–aptamer recognition. (A) Principle of detection of thrombin–aptamer recognition in a nanochannel array. (B) Schematic diagram of the *I–V* measurement setup. (C) The *I–V* properties of PAA under different conditions with a scan rate of 100 mV/s. Blue line: TBA-modified nanochannel array; red line: after the addition of thrombin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

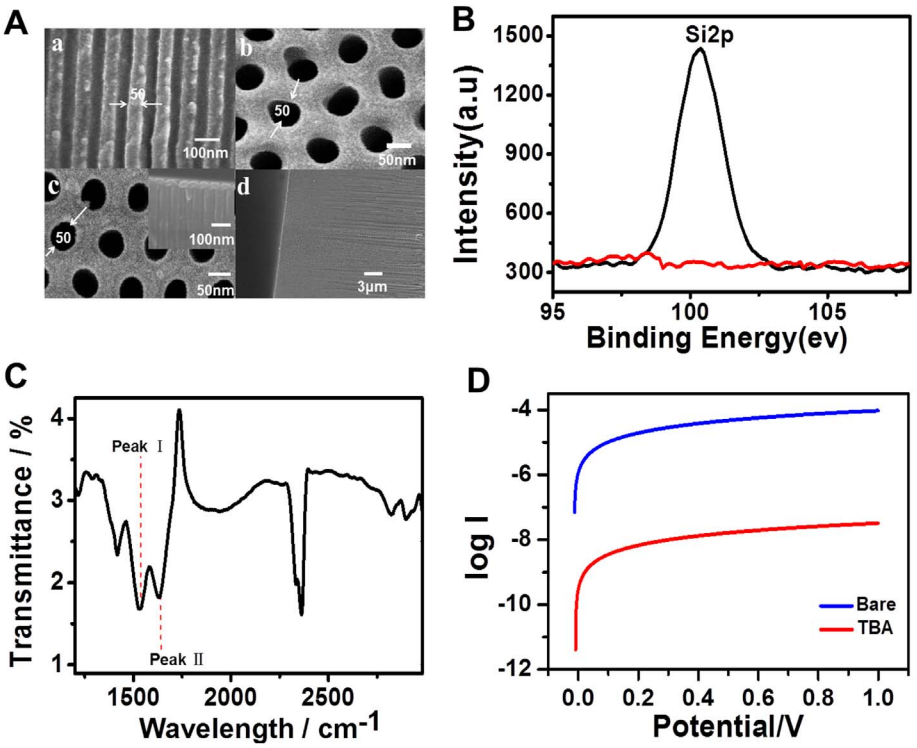

Fig. 2. (A) SEM images of (a) a cross-section; (b) the top; (c) the bottom; (d) the whole cross-section of the PAA membrane (the inset of c is the barrier layer of the PAA before detachment). (B) XPS spectra of pure PAA (red line) and APTMS-modified PAA (black line). (C) FTIR spectrum of TBA-immobilized PAA using pure PAA membrane as the reference. (D) Plots of (lg *I*) vs potential for the bare PAA (blue line) and TBA-modified PAA (red line) from 0 to 1 V. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)