A Label-Free and Rapid Molecular Biosensor Based on the Combination of the Extended Gate field Effect Transistor and AC Electrokinetics

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Abstract
In this study, we present a label-free and rapid molecular biosensor based on the combination of the extended gate field effect transistors (EGFET) and AC electroosmosis (ACEO). The ACEO significantly promotes the sensitivity and reaction rate of EGFET-based molecular biosensor that can achieve 0.5 ng/ml sensitivity and minute-level detection time. The results show that ACEO-EGFET not only significantly reduces the detection time of antibody-antigen binding from hours to a few minutes, but also effectively lowers detection limit by 100 times to reach sub-ng level sensitivity, which is compared to the conventional incubation method. This rapid, portable and highly sensitive platform is very applicable to be used for field-use detection.

1. Introduction
In recent years, immunoassay plays an important role in the prevention of infectious disease spread and pathogens. Therefore, researches had been more interesting in development of immunoassaysensors for detection of sub-nM concentration of biomolecules in a few minutes [1, 2]. In order to realize highly sensitive and rapid of immunoassay, enzyme-linked immunosorbent assay (ELISA) integrated with real-time transducer had been the most widely adopted technique because it can achieve high sensitivity, but it still requires a much longer analysis time due to the antibody-antigen binding is limited by the diffusion-dominated kinetics. For example, Chen et al [3] demonstrated a GaN nanowire based EGFET for DNA detection, which has wide detection range, 10^-19 to 10^-6 M, but DNA-DNA docking still needs over 30 min. While, substantial studies have been performed pico-Molar sensitivity by improving the performance of transducers. For a very dilute molecular solution, the low diffusion coefficient and long diffusion length are still major factors that cause the hour-level detection time. Therefore, rapid concentration of analyte techniques were integrated into immunoassays developed and attracted much attention in many biochemical applications [4,5]. AC electroosmosis (ACEO) is generated by the electric field-induced ion migrations drag the fluid to form a bulk flow over the electrodes. The evident ACEO occurs at frequencies below the charge relaxation frequency of the analyte solution. The field-induced fluid would accelerate the molecules binding on the detection surface in a short time.

In this investigation, utilizing the combination of EGFET and ACEO to develop a minute-level detection time and sub-ng/ml sensitivity for application on protein A detection. Moreover, comparing incubation method, the EGFET with ACEO concentration not only significantly reduces detection time by 100 times but also effectively improves the sensitivity to reach 0.5 ng/ml.

2. Results and Discussion
The diagram of AC electroosmosis enhanced Extend Gate field effect Transistors (ACEO-EGFET) shown in Fig. 1(a), which includes two modes: One is measurement mode, when working electrode (WE) was connected to gate of commercial n type metal-oxide-semiconductor field-effect-transistor (n-MOSFET; CD4007) as an extended gate. All of characteristic of I_DSS-V_ref were obtained in measurement mode with 0.001×PBS solutions, where V_DSS was fixed at 0.1 V and swept voltages from -1.5 V to 3V was applied on reference electrode (RE), Ag/AgCl (Sat. NaCl). Another is concentration mode (switching connected to "2") that is used for concentrating and trapping protein A on WE by electokinetic forces, In Fig. 1(b). The IgG immobilization on WE has six functionalization steps, shown in Fig. 2(a) and listed as follow: (1) Cleaning WE-surface by piranha solution; (2) Self-assembly monolayer of 11-MUA; (3) Functionalization by EDC/NHS; (4) IgG immobilization; (5) Blocking of the reactive immobilization sits by BSA; (6) detection of protein A. The functionalization steps were detected by EGFET, shown in Fig. 2(b).

To compare the characteristic of ACEO and conventional ELSA method, in the detection steps, the WE is immersed in PBS with various concentrations of protein A to form incubation-EGFET. The response time of ACEOC-EGFET compared to incubation were shown in Fig. 3. The ACEO-EGFET in 5 ng/ml protein A has response at 5min and near saturated at 8 min, which is much faster than incubation-EGFET, 60min and near saturated at 90min. In Fig. 4, the ACEO-EGFET and incubation in var-
ious concentrations of protein A was further discussed. The sensitivity of ACEO-EGFET is 57.1 mV/(ng/ml) and 30.9 mV/(ng/ml) in detection range of 0.5-5 ng/ml and 5-10 ng/ml, respectively. The performance of ACEO-EGFET is greater than incubation-EGFET, 0.4 mV/(ng/ml) range of 50-200 ng/ml. The results are demonstrated that the ACEO-EGFET not only significantly reduce detection time, from many hours to couple of few minutes, but also effectively improves 100 times of sensitivity and realizes sub-ng level sensing region.

![Fig. 1](image1.png)

Fig. 1 (a) The structure of ACEO-EGFET. RE is reference electrode; WE is working electrode; CE is concentration electrode. $V_{DS}$ is voltage supplied to drain, $I_{DS}$ is the current of drain-source, and $V_{Ref}$ is swept voltage supplied to RE. (b) Protein A are carried to the WE by ACEO.

![Fig. 2](image2.png)

Fig. 2 (a) Schematic illustration of the IgG immobilization on WE-surface. (b)The shift of characteristic $I_{DS}$-V$_{Ref}$ in each immobilization steps (cleaning, immobilization, blocking, and detection).

![Fig. 3](image3.png)

Fig. 3 Response time of ACEO-EGFET and Incubation-EGFET.

![Fig. 4](image4.png)

Fig. 4 ACEO-EGFET and Incubation-EGFET in various concentrations of protein A.

3. Conclusions

An ACEO-enhanced EGFET biosensor has been developed and well demonstrated in this study. The enhancement of the detection time and sensitivity were also investigated by comparing ACEO-EGFET with the conventional incubation EGFET. The result shows that ACEO can accelerate the reaction rate to allow molecules binding on the detection surface of EGFET rapidly and finish the detection in only 5 min. The sensitivity is as high as 57.1 mV/(ng/ml) and 30.9 mV/(ng/ml) in detection range of 0.5-5 ng/ml and 5-10 ng/ml, respectively. The detection limit was reduced 100 times and the detection time was reduced from 90 min to 5 min. Thus, ACEO-EGFET not only significantly reduces detection time but also effectively improves the sensitivity and sensing region. This rapid and portable positive–negative diagnostic platform would be a preliminary screening step for many applications such as urethral irritation and environmental monitoring in field.

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