Enhanced Electrical Label-Free Detection of Pathogens Through Isothermal DNA Amplification Using True Dual-Gated ISFETs

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We present a novel structure of ion-sensitive field-effect transistor (ISFET) that can be operated in a 'tailored dual-gate' mode where the transistor's pH signal-to-noise ratio (SNR) is larger than for the traditional configurations. The increased SNR enables the resolution of smaller pH changes enabling more accurate monitoring of biological reactions. When used to detect isothermal DNA amplification for identification of pathogens, the new transistors differentiate positive from negative samples in shorter detection times and providing a greater signal. These studies demonstrate that the novel ISFET architecture and the dual-gate operation can lead the way for electronic-based label-free portable diagnostic tools.

Introduction: Detection of biochemical reactions that provide small signals have driven research toward signal enhancement techniques for ISFET sensing. For reactions where the measured variable is the solution's pH, like DNA amplification, a number of publications demonstrated the use of coupled transistors for signal enhancement. Dual-gated transistors operated in parallel yield a "super-Nernstian" sensitivity that exceeds the 59 mV/pH limit. Here we present an improved dual-gate FET, fabricated in a semiconductor foundry with a 0.18μm SOI technology. The new transistor has an individually addressable back-gate which allows a tailored dual-gate operation that result in an enhanced SNR for pH changes that allows a faster detection of pathogenic genes.

Materials and Methods: The transistor is fabricated by Taiwan Semiconductor Manufacturing in a two-stage SOI process that yields a dual-gate ISFET with individually addressable back-gate (schematic of the structure in Fig.1 inset). The sensor pH response was evaluated both for the dual- and single-gate operation modes taking transfer characteristics with electrolytes of different pH. This characterization was repeated for multiple fluid-gate biases to optimize the sensor's SNR. The transistor is then used in the single- and dual-gate operation modes to monitor pH changes caused by a loop-mediated DNA amplification (LAMP) reaction of pathogenic *E.Coli* O111.

Results and Discussion: The ISFET's single-gate operation exhibits the common linear relation between surface potential and pH, having a sensitivity of 52.91 mV/pH. On the other hand, the dual-gate mode shows a nonlinear response to pH with dynamic sensitivity and noise. By choosing appropriate biasing conditions and pH ranges, the dual-gate operation has sensitivity above the Nernst limit without significant noise increments. The improved SNR enhances the resolution from 0.03 to 0.015 resolvable pH units. This improved resolution allows a faster detection of LAMP. The dual-gate mode can detect the amplification of pathogenic genes 10 min faster than the single-gate mode (Figure 1). The increased SNR is explained by the dominance of extrinsic sources of noise that are not being amplified by the increased sensitivity.

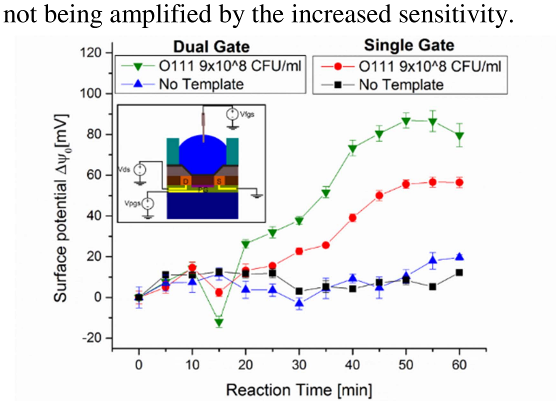

Figure 1. ISFET detection of LAMP DNA amplification targeting the *wzy* gene of *O111 STEC.* Change in surface potential as a function of reaction time for the single- and dual-gate operation modes for a positive sample with template DNA where amplification is expected and a negative control without template. The inset is showing a schematic of the dual-gated ISFET showing the applied potentials where 'D' represents drain, 'S' Source, 'PG' poly-gate, and 'FG' fluid-gate node.

Conclusions: The pH changes related to incorporation of nucleotides in a DNA amplification reaction are better monitored with an ISFET in a tailored dual-gate operation. At specific biasing conditions the noise is being dominated by an extrinsic source and the greater sensitivity of the dual-gate mode improves the sensor resolution. This enables tracking smaller pH changes which result in faster reaction detection times. The new structure has particular potential for applications targeting point-of-care diagnosis that are subject to noisy environments and require a fast turnaround.

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