

Boronate probe-based hydrogen peroxide detection with AlGaIn/GaN HEMT sensor

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Impact statement

This study presents a novel application of a surface sensitive electronic sensor made up of a biocompatible semiconducting material system (AlGaIn), for the detection of reactive oxygen species present in biological settings that contain hydrogen peroxide. The results from this study demonstrate the possibilities of interfacing biocompatible electronics with existing bioorthogonal probes to expand the capabilities of existing detection techniques. Additionally, this opens new avenues for the development of future hybrid electro-chemical sensing systems which can interface with living systems and allow real-time remote monitoring.

Abstract

The results from this study demonstrate the potential of an AlGaIn/GaN high electron mobility transistor sensor for the detection of reactive and transient biological molecules such as hydrogen peroxide. A boronate-based fluorescent probe was used with this device to detect the presence of micromolar levels of hydrogen peroxide typically associated with intracellular processes. The real-time electrical response of the high electron mobility transistor sensor showed a gradual decrease in the two-dimensional electron gas current as the reaction proceeded over time. A corresponding increase in the emission intensity was measured from the fluorescent probe with the progression of the reaction. The fluorescence from the boronate probe was used as an indicator to confirm the detection of hydrogen peroxide. These results demonstrate the dynamic measurement capability of AlGaIn/GaN high electron mobility transistor sensors in monitoring real-time reactions of reactive oxygen species such as hydrogen peroxide.

Keywords: Hydrogen peroxide, AlGaIn/GaN HEMT, ROS, HEMT sensors, boronate probe, electrochemical sensing

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Introduction

Over the last three decades, a tremendous amount of research has been conducted in the development of the III-nitride (III-N) material system and device technology.^{1–13} III-N-based AlGaIn/GaN high electron mobility transistors (HEMTs) have been extensively studied for biochemical sensing applications due to their high sensitivity to surface phenomena, fast response time, aqueous stability, and good biocompatibility.^{14–17} In HEMT sensors, the two-dimensional electron gas (2DEG) acts as a highly conductive channel that can be modulated by changes in the surface potential. The detected signal in the gate region is amplified and measured as a change in the 2DEG conductivity, which makes the HEMT a good sensing device. Based on this principle, HEMT sensors have demonstrated promising results in the detection of pH changes,^{18,19} gas molecules,^{20–22} DNA,^{23,24} and cancer-associated antigens,^{25,26} as well as in monitoring living cells.^{27,28} Due to their unique electrical and chemical properties, the AlGaIn/GaN HEMTs show great potential for the

detection of reactive and transient biological components, such as reactive oxygen species (ROS). Amongst the various ROS molecules, hydrogen peroxide (H₂O₂) is widely studied, as it is relatively stable and abundant in comparison to other ROS molecules. H₂O₂ also has been implicated in various roles, both beneficial and detrimental to cellular health.^{29–31} As a beneficial species, it is used as a signaling molecule to regulate various cellular processes. On the other hand, high levels of H₂O₂ have been shown to cause oxidative stress, which is associated with damage to cellular structures, aging, neuro-degenerative diseases, and the formation of malignant cells. To understand the complex roles played by H₂O₂, several techniques have been developed to detect and monitor it in its native settings with high selectivity and sensitivity.^{32–35} One of the conventional approaches for detection involves the use of fluorescent probes which react specifically with H₂O₂.^{36,37} Though such a method is reliable, it is time-consuming and involves extensive processing. To overcome limitations of the conventional approaches while utilizing their

advantages, there is a strong interest in the development of hybrid electro-chemical sensing systems, as detection based on electron transport can generate larger and faster signals.²⁸ Additionally, electro-chemical detection offers the potential advantage of real-time and remote monitoring through the development of integrated sensing platforms. In this letter, we report the use of an AlGaIn/GaN HEMT-based sensor for detection of the presence of H₂O₂ by correlating its measured change in conductivity with a reaction of H₂O₂ and a boronate-based fluorescent probe in the gate-well region. Here, we utilize the high selectivity of the boronate probe to detect H₂O₂, and the surface sensitivity of HEMTs to generate a corresponding electrical response. A non-functionalized, non-metallized open-gate AlGaIn/GaN HEMT sensor is employed in this study. The real-time progression of the reaction is monitored by measuring the modulation of the 2DEG current in response to changes in the surface potential caused by the interaction between the semiconductor surface states and the products of the reaction. The response of our HEMT sensor shows its applicability to the detection of micro-molar concentrations of H₂O₂, which is in the range typically present in intracellular processes of living systems.^{29,31,36,38}

Materials and methods

The AlGaIn/GaN HEMT sensor employed in this study was fabricated on a HEMT structure consisting of a ~4 μm thick nominally undoped GaN buffer layer, 20 nm thick Al_{0.3}Ga_{0.7}N barrier layer, and 4 nm thick Si-doped n⁺GaN cap layer. The epilayers were grown by metal organic chemical vapor deposition on a 2-inch sapphire substrate.^{39–41} Hall effect measurement on this structure showed a 2DEG density of $1.43 \times 10^{13} \text{ cm}^{-2}$, carrier mobility of 1060 cm²/V-s, and sheet resistance of 418 Ω/sq. Mesas roughly 250 nm in height were formed by inductively coupled plasma etching using low power BCl₃/Cl₂/Ar chemistry to isolate active device area from rest of the substrate. Source and drain ohmic contacts consisting of Ti (15 nm)/Al (60 nm)/Mo (35 nm)/Au (80 nm) multilayer stacks were deposited and annealed at 850°C in nitrogen ambient for 30 s. SU-8 negative photoresist was used to encapsulate the source/drain contacts and to create a well-type region in the gate area for liquid testing. The dimension of the gate-well region was 100 μm × 140 μm. An optical image of the fabricated device is shown in Figure 1. Electrical conductivity between the source and drain terminals was measured at room temperature using an Agilent B1500A semiconductor device parameter analyzer.

The boronate-based fluorescent probe Peroxy Orange 1 (PO1) was employed for the detection of H₂O₂.^{34,36} Boronate probes are bioorthogonal and well-established for the detection and imaging of H₂O₂ in biological settings with high selectivity and sensitivity.³⁴ Selectivity tests have shown negligible turn-on of boronate probes in the presence of other ROS molecules. These probes undergo an irreversible reaction into a fluorescent state upon reaction with H₂O₂, which is advantageous in detecting transient and low levels of H₂O₂ in living systems. From previous reports, the boronate probes can reliably detect H₂O₂

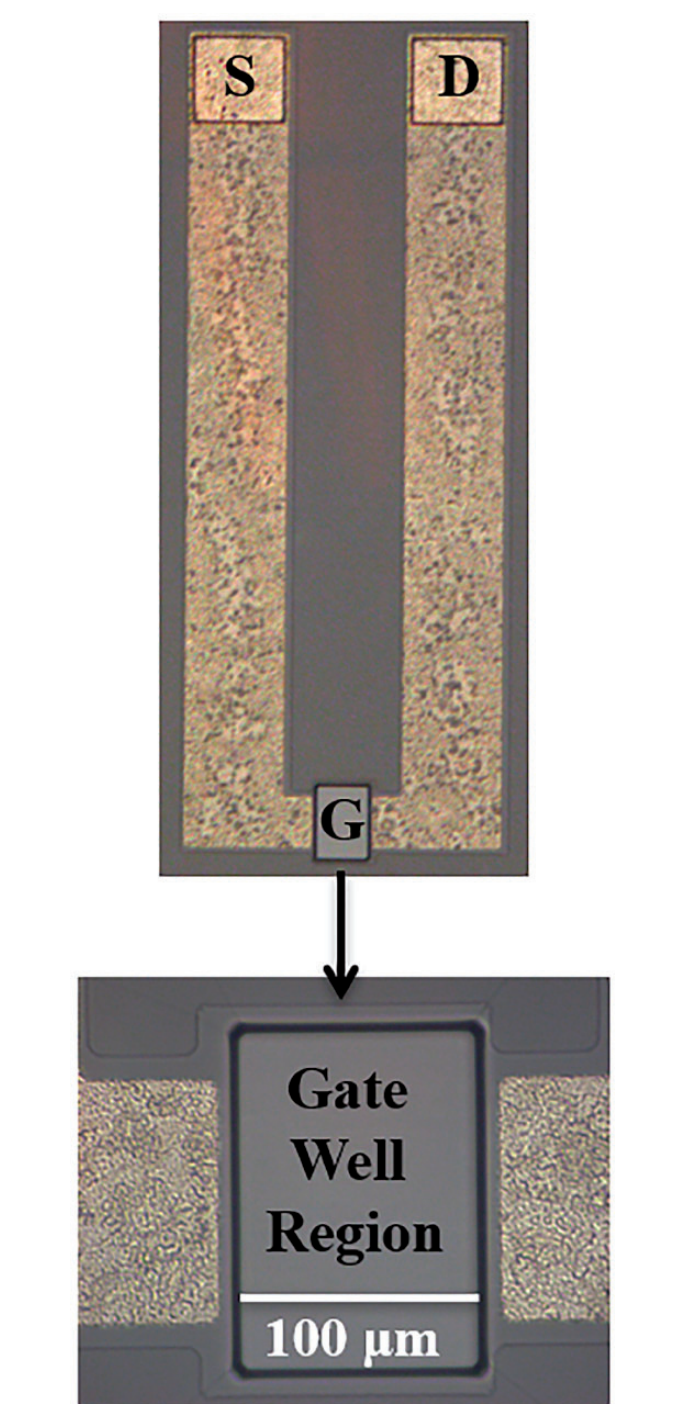


Figure 1. Optical image of fabricated AlGaIn/GaN HEMT sensor. (A color version of this figure is available in the online journal.)

concentrations as low as tens of micromolar to hundreds of nanomolar in aqueous solution.⁴² While the presence of an optical transition is required for the fluorometric usage of these probes, it is not required for electrical detection where factors such as charge, and adsorption behavior are more important. In this study, the charge associated with the reaction products is utilized to generate a corresponding electrical response. Additionally, the ability to measure reaction progress through fluorescence allows for the comparison of optical and electrical detection of

the reaction. The reagents, H_2O_2 and PO1, were obtained from Sigma Aldrich. Dilutions of H_2O_2 were prepared on the days of testing by diluting a stock (35% by weight) in deionized water to obtain fresh solutions containing micromolar concentrations of H_2O_2 . In this study, anhydrous dimethyl sulfoxide (DMSO) was used as a solvent to prepare solutions of the boronate probe PO1, as it is a known standard for this type of reagent.³⁶ Previous reports indicate that DMSO shows almost negligible reactivity with H_2O_2 under the current test conditions.^{43,44} During testing, appropriate volumes of this solution were added to dilute aqueous H_2O_2 to obtain a probe concentration of $5\ \mu\text{M}$ in the final solution, as per the manufacturer instructions.

Electrical measurements were performed after transferring the test solution into the gate-well region of the device via a microfluidic line. Source-to-drain channel current (I_{DS}) measurements were conducted to investigate how the 2DEG conductivity changed as a function of time. A low drain-source voltage ($V_{\text{DS}} = 100\ \text{mV}$) was utilized to prevent any electrochemical reactions and to minimize the heat generation in the device associated with high current densities. Measurements were performed in a dark environment at room temperature. Initial electrical measurements with aqueous H_2O_2 solutions of varying concentration confirmed that the 2DEG current does not change with the addition of dilute peroxide alone. This is expected, because the detection mechanism of an AlGaIn/GaN HEMT sensor depends on the electrostatic interaction between the analytes and electronic surface states of the device. As such, neutrally charged molecules such as H_2O_2 are not expected to generate a strong response. Molecules with specific reactivity towards H_2O_2 such as the boronate probes which are used in this study, generate charged products upon reaction with H_2O_2 which enables indirect detection. Baseline measurements were first performed by testing DMSO+PO1+ H_2O solutions not containing any H_2O_2 . Subsequent measurements were performed after adding the DMSO+PO1 mixture to the H_2O_2 dilutions. To ensure homogeneous composition, the solutions were mixed well before transfer onto the gate-well region. Once the reaction was started, the real-time response of HEMTs was monitored for 200 s intervals at different points in the reaction.

Results

The electrical response of the HEMT sensor to test solutions initially containing $25\ \mu\text{M}$ and $12.5\ \mu\text{M}$ concentration of H_2O_2 with $5\ \mu\text{M}$ boronate probe solution deposited in the gate-well region is shown in Figure 2(a) and (b),

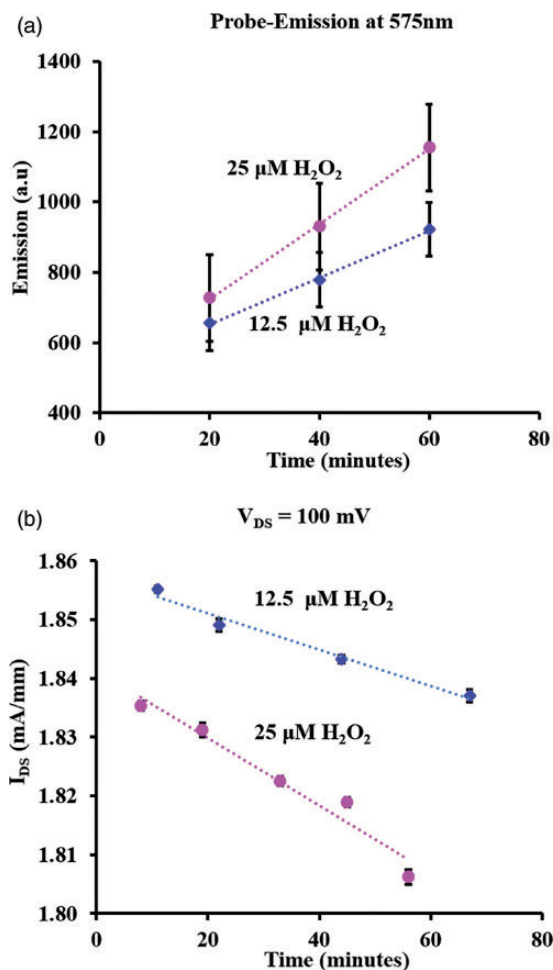


Figure 3. (a) Fluorescence emission over time, and (b) electrical measurements showing real-time change in 2DEG current for H_2O_2 solutions of $25\ \mu\text{M}$ (magenta trend line with circular markers) and $12.5\ \mu\text{M}$ (blue trend line with diamond markers). The dashed lines between points are a guide for the eye. (A color version of this figure is available in the online journal.)

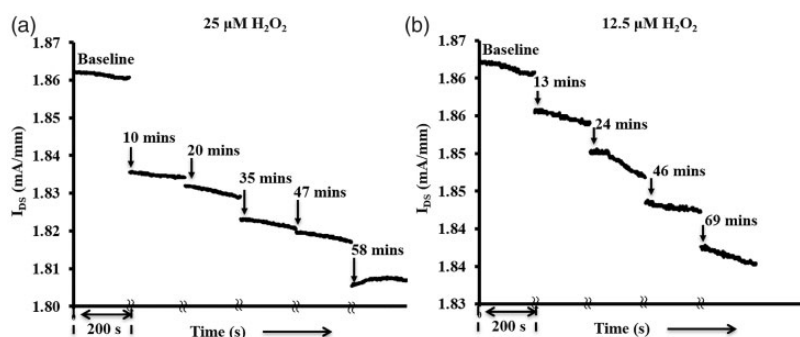


Figure 2. 2DEG channel current measurement as a function of time at $V_{\text{DS}} = 100\ \text{mV}$ with (a) $25\ \mu\text{M}$ and (b) $12.5\ \mu\text{M}$ of H_2O_2 . The real-time response of the device was tested for 200 s, first with baseline solution (DMSO + PO1 + H_2O), and then at different reaction times after the addition of H_2O_2 to the baseline solution.

respectively. From the figure, for a fixed concentration of the boronate probe ($5\ \mu\text{M}$), a higher rate of decrease in 2DEG current is observed in the test with the higher concentration of H_2O_2 . A decrease in the 2DEG current by $0.029\ \text{mA}/\text{mm}$ and $0.018\ \text{mA}/\text{mm}$ was measured after the reaction had proceeded for about an hour with solutions initially containing $25\ \mu\text{M}$ and $12.5\ \mu\text{M}$ of H_2O_2 , respectively.

To confirm the expected reaction between PO1 and H_2O_2 , as well as to characterize the rate of the reaction, the fluorescent emission of the reaction mixture was measured at various intervals as the reaction progressed with a FlexStation-3 fluorescence plate reader from Molecular Devices. Maximum emission of the probe was measured at $575\ \text{nm}$ wavelength with an excitation wavelength of $540\ \text{nm}$. Figure 3(a) shows the intensity of the emission from the activated fluorescent probes generated upon reaction with H_2O_2 , measured by the fluorescence plate reader. The intensity of the emission increases with the concentration of H_2O_2 , which is in agreement with previous reports.³⁷ Comparing the trends from the optical (Figure 3(a)) and the electrical measurements (Figure 3(b)), it can be seen that as the reaction proceeds, an increase in the emission intensity and a corresponding decrease in 2DEG current is observed. For a fixed probe concentration, emission intensity is higher for solutions with higher initial concentration of H_2O_2 , which corresponds with a larger decrease in the 2DEG current.

Discussion

The sensing mechanism of the HEMT device involves detection of the changes in the surface potential correlated

with the reaction between H_2O_2 and the boronate probe. From the electrical characterization data, a steady decrease in the 2DEG channel current was measured over time as the reaction proceeds. It is well known that changes in the charge accumulated at the liquid/semiconductor interface directly affect the surface states of the semiconductor. Due to this, the electrostatic compensation which exists between the surface states and 2DEG is unbalanced, which results in modulation of the 2DEG current.^{45–47} The reaction between the boronate probe and H_2O_2 molecules results in the release of an activated fluorescent probe and a weakly acidic pinacol boronic acid, as can be seen in Figure 4. The real-time electrical response of the 2DEG current, shown in Figure 2, indicates accumulation of net negative surface charge at the liquid/semiconductor interface as the reaction progresses. The accumulation of negative surface charge is attributed to the interaction of the delocalized Π electrons and charged carboxylate group in the activated boronate probe with the surface states of HEMT. As more products are generated, a gradual decrease in the 2DEG current is observed over time, as illustrated in Figure 4(b). Comparing the rate of change in the emission and electrical response shown in Figure 3, increasing the initial concentration of H_2O_2 from $12.5\ \mu\text{M}$ to $25\ \mu\text{M}$ amplifies the rate of increase in the emission by a factor of 1.6 and the rate of decrease in the 2DEG conductivity by a factor of 1.9, which suggests higher sensitivity of the electrical response to H_2O_2 detection in this range. These trends further demonstrate the ability of HEMT sensors in detecting H_2O_2 at concentration levels typically associated with intracellular processes. Additional work focused on functionalization of the gate region will enhance the selectivity of the

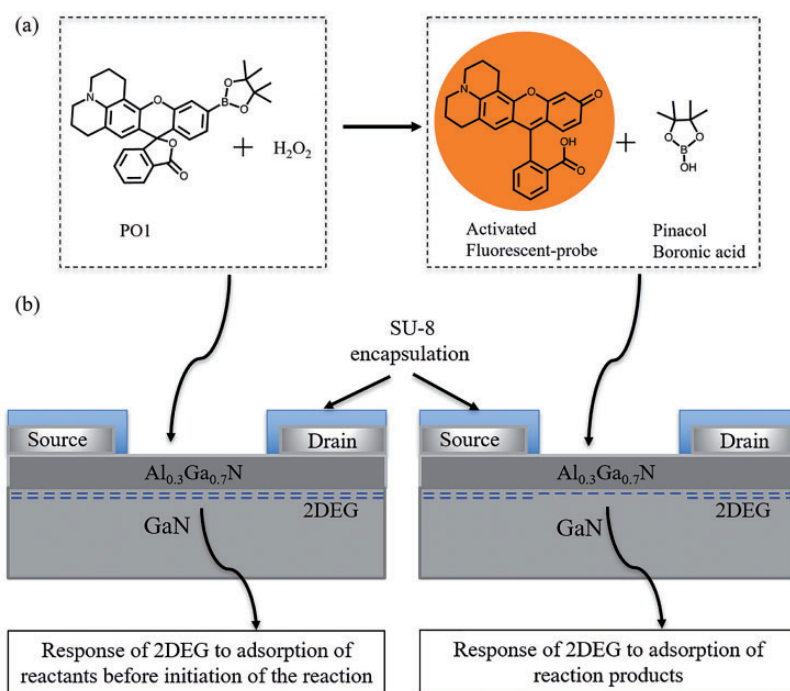


Figure 4. (a) Illustration of the reaction between boronate probe PO1 and H_2O_2 (Adapted from Lippert *et al.*³⁴ and Lin *et al.*³⁶). The reaction results in the release of an activated fluorescent probe and a weakly acidic pinacol boronic acid. (b) Schematic representation of 2DEG modulation to the adsorption of reactants (left) and reaction products (right) to the HEMT surface. (A color version of this figure is available in the online journal.)

sensor in biological settings in the presence of other charged molecules, enabling *in vitro* utilization.

Conclusions

An AlGaIn/GaN HEMT sensor was used in the present work to detect H₂O₂ using a boronate-based fluorescent probe. The sensitivity of the HEMT surface to changes in the surface potential was utilized to successfully measure the progress of the reaction between a boronate-probe and H₂O₂. Observations from this study confirm the dynamic sensing capability of HEMT sensors in monitoring real-time changes which occur during the reaction between a ROS molecule, such as H₂O₂, and a molecule with specific reactivity. The biocompatible and stable nature of the III-N material system opens new possibilities for the development of AlGaIn/GaN HEMT sensors which can interface with living cells to understand the complex functionality of ROS molecules in their native settings.

AUTHORS' CONTRIBUTIONS

IM and RJR performed experiments, measurements, data collection, analysis, visualization, and writing of the article; BM participated in formal analysis, discussion, and review of the article; KH and ER contributed towards maintenance and support with semiconductor material growth; JAM, NCC and FSS were involved in supervision, methodology, resources, validation, and review of the article.

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