

Fabrication and characterization of a biologically sensitive field-effect transistor using a nanocrystalline diamond thin film

Wensha Yang and Robert J. Hamers^{a)}

Department of Chemistry, University of Wisconsin—Madison, 1101 University Avenue, Madison, Wisconsin 53706

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We report the fabrication and characterization of a biologically sensitive field-effect transistor (Bio-FET) using a nanocrystalline diamond thin film. Biomolecular recognition capability was provided by linking human immunoglobulin G (IgG) to the diamond surface. Electrical measurements reveal behavior characteristic of field-effect transistors. The biomolecular recognition and specificity characteristics were tested using the two antibodies anti-IgM and anti-IgG. Electrical measurements show that the Bio-FET device made on an IgG-modified diamond exhibits a response specific to the anti-IgG antibody. Our results demonstrate the ability to fabricate a bio-FET device using a biologically modified diamond thin film. © 2004 American Institute of Physics.
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While the unusual mechanical, thermal, and electrical properties of diamond have long been studied,^{1–3} it is only recently that the outstanding chemical properties of diamond^{4,5} have made it an attractive substrate for biological studies,^{6–8} and especially the integration of biological systems with microelectronics.⁹ Silicon-based field-effect transistors (FETs) can be provided with chemical and/or biological sensitivity by modifying the gate insulator with specific molecular layers.^{10–13} However, the chemical instability of silicon in aqueous media limits its utility for bioelectronic devices.^{14–16} Diamond surfaces that have been covalently modified with biomolecules have extremely good chemical stability and biological selectivity.^{6–8} The biomolecular recognition properties also alter the electrical properties of the diamond via a field effect, which can be detected via changes in the interfacial impedance,^{9,17} suggesting the possibility of fabricating biologically sensitive field-effect transistors (Bio-FETs) on diamond similar to the ion-sensitive FETs prepared previously,^{18,19} but with the added benefit of biomolecular recognition capability. Here, we report the construction and characterization of a bio-FET device from diamond thin films for direct detection of biological molecules.

Figure 1 shows an overview of the fabrication process. Figure 1(a) shows the chemical modification, while Fig. 1(b) shows the physical structure of the FET, which is similar to that of the classical ion-sensitive field-effect transistor structure.¹⁰ Undoped nanocrystalline diamond thin films (1 μm thick) were grown on *n*-type silicon (10 $\Omega\text{ cm}$) substrates by microwave-enhanced plasma chemical vapor deposition. The diamond film must be pinhole free to protect the silicon substrate from chemical attack, but must be thin to minimize gate leakage and provide optimal sensitivity. This combination of properties was achieved by mechanically abrading the silicon surface using diamond powder (0–2 μm diameter) to provide a very high initial nucleation density.¹ Scanning electron microscopy images after growth showed a smooth, continuous diamond film with grain sizes of 20–100 nm.

To provide biomolecular recognition capability, the diamond surfaces were modified with an antibody, human immunoglobulin G (IgG), through a multi-step process shown in Fig. 1(a). The diamond surfaces were first terminated with hydrogen using a radio-frequency plasma, and an organic monolayer film bearing protected amine groups (10-aminodec-1-ene, protected with trifluoroacetic acid) was covalently linked (step 1) to the surface via photoexcitation at 254 nm.²⁰ De-protection in 0.36 M HCl/methanol (step 2) followed by reaction with 3% solution of glutaraldehyde in a sodium cyanoborohydride coupling buffer for 2 h (step 3) then produced a diamond surface with some exposed aldehyde groups. Gold was sputter coated on the aldehyde-terminated diamond surfaces through a mask to form ohmic source and drain contacts,²¹ leaving a channel region 0.5 mm in length and 3 mm in width. The metal electrodes were then covered with a thin layer of epoxy to insulate them from the solution. Finally, the exposed, aldehyde-modified surfaces were immersed in a 1 mg/ml human immunoglobulin G (IgG) solution for approximately 8 h (step 4) which binds the IgG to the surface. Unreacted aldehyde groups were stabilized against further reaction by immersing in a solution of 0.1 M glycine in sodium cyanoborohydride buffer. Although the number of IgG molecules bonded to the surface was not measured directly, based on the molecular dimensions we expect a maximum of $\sim 1 \times 10^{12}$ molecules/cm².

To complete the FET structure, a flow cell consisting of a polydimethylsiloxane sheet with embedded microfluidic channels was used to separate the diamond surface by 1 mm from a Pt gate electrode.¹⁰ The flow cell was filled with de-ionized water for measurements and with various electrolyte solutions containing biomolecules of interest to test the biomolecular recognition capabilities. All electrical measurements reported here were performed in 18 M $\Omega\text{ cm}$ de-ionized water.

Figure 2(a) shows the changes in drain-source current (I_{ds}) as a function of drain-source voltage (V_{ds}) for different values of gate bias (V_{gs}) of an IgG-modified diamond FET. Even when $V_{\text{ds}}=0$ there is a small drain current due to leakage from the gate; this leakage current has been subtracted from the original data before plotting in Fig. 2(a). For each

^{a)} Author to whom correspondence should be addressed; electronic mail: rjhamers@wisc.edu

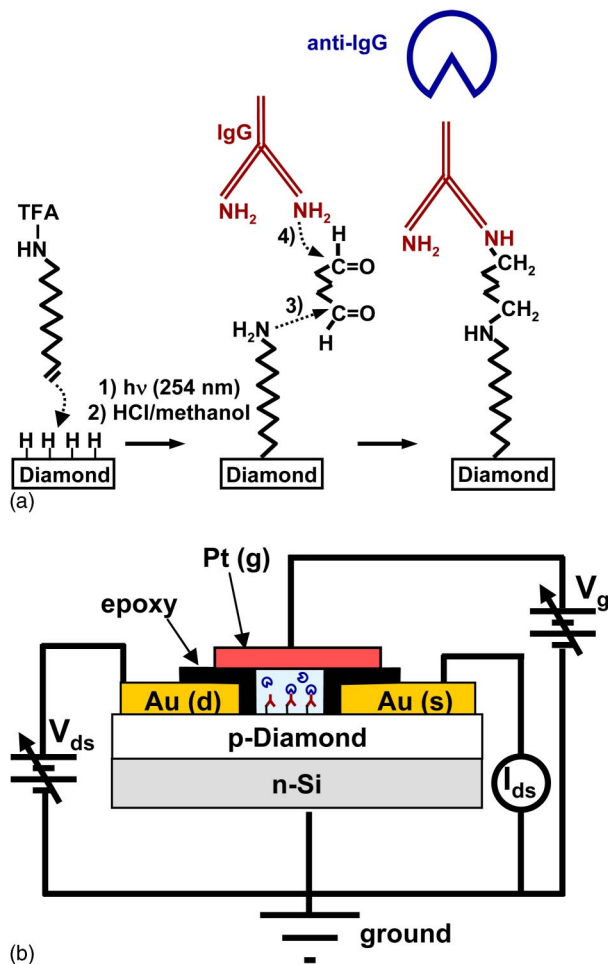


FIG. 1. (Color online) (a) Overview of the chemistry for linking human IgG to diamond surfaces. (b) Schematic illustration of diamond Bio-FET.

value of V_{gs} , the drain current increases with V_{ds} and then begins to saturate. This saturation behavior is similar to that observed for more conventional silicon-based FETs.²² As a control experiment [Fig. 2(a) dashed line], this device was also exposed to a buffer solution containing 68 $\mu\text{g/ml}$ anti-human IgM (anti IgM), an antibody that does *not* bind to IgG but which otherwise has similar properties. The anti-IgM-containing buffer solution acts as a control to verify that changes induced by exposure to the specific antibody anti IgG (to be presented below) are a consequence of a specific biomolecular recognition process. The data in Fig. 2(a) show that the electrical properties before and after exposure to the IgM-containing buffer solution are nearly identical, indicating that the buffer solutions and nonbinding anti-IgM antibody do not affect the electrical properties.

Figure 2(b) shows the drain current versus gate voltage for two different values of the drain-source voltage. Beyond a threshold of ~ -1 V, the current increases approximately quadratically with gate voltage. Measurements made in the reverse direction are nearly identical. These results show that I_{ds} depends on the gate voltage in a manner typically observed for FETs.²²

To verify that the current response observed is primarily from the channel formed in the diamond, several control experiments were performed. Measurements of the Au/diamond/n-Si interface showed highly rectifying behavior, indicating that the reverse-biased junction of n-Si and p-diamond confines the current flow to the diamond. Addi-

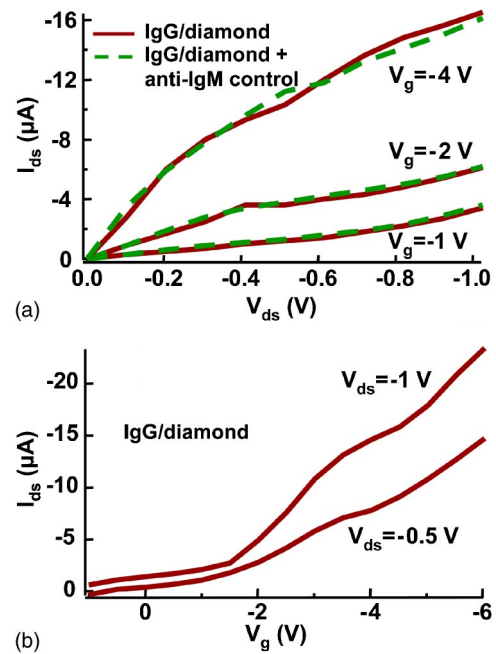


FIG. 2. (Color online) (a) I_{ds} - V_{ds} curves at different gate voltages for IgG modified diamond surface before (solid line) and after (dashed line) exposure to anti IgM. Measurements were performed in de-ionized water. (b) I_{ds} - V_g curves of IgG modified diamond surfaces with $V_{ds}=-0.5$ V (dashed line) and $V_{ds}=-1$ V (solid line).

tional measurements showed that the leakage current through the epoxy protecting layer coating the Au electrodes was typically nanoamperes in magnitude, far smaller than the microampere drain currents observed in the FET measurement. To minimize the current leakage through the solution, which is in parallel with the channel, the measurements were performed in the de-ionized water, yielding an estimated >90 M Ω solution resistance between electrodes. From Fig. 2(a), the overall resistance between the drain and the source electrodes can be calculated from the slope of the linear region of the I_{ds} - V_{ds} curve, yielding a value of 40 k Ω with $V_g=-4$ V. Since this value is much smaller than the solution resistance, we conclude that the drain-source current flow is almost entirely through the channel formed in the diamond.

To demonstrate the ability to respond selectively to the anti-IgG antibody, the electrical properties were measured before and after exposure to anti IgG (134 $\mu\text{g/ml}$), an antibody which binds strongly to the surface-tethered IgG. Figure 3(a) shows the drain current versus drain-source voltage for an IgG-modified diamond FET at a gate-source voltage of -4 V (solid line). After exposure to anti IgM (68 $\mu\text{g/ml}$, 15 min incubation time) as a control, the response (dashed line) remains unchanged. However, the dotted line shows that after exposure to anti-IgG (134 $\mu\text{g/ml}$, 15 min incubation) there is a pronounced reduction in drain current. At a gate voltage of -4 V, the drain current decreases by approximately 25%. The biomolecular recognition between the IgG-modified surface and the solution-phase anti IgG can also be detected via the changes in drain current as a function of the gate voltage, as shown in Fig. 3(b). As expected, the ability to detect the selective binding is most apparent at the largest negative gate voltages; at $V_g=-6$ V, the binding of anti IgG to the surface induces a change of approximately 30% in the drain current.

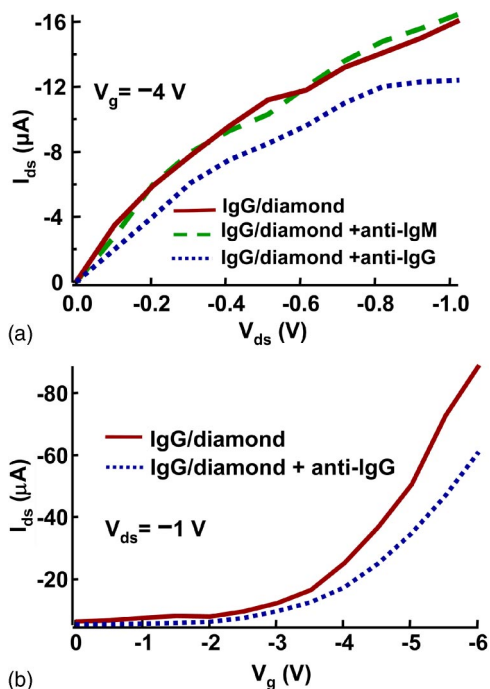


FIG. 3. (Color online) (a) I_{ds} - V_{ds} curves at $V_g = -4$ V for a IgG modified diamond FET before (solid line) and after exposure to anti IgM (dashed line) and to anti IgG (dotted line). (b) I_{ds} - V_g curves at $V_{ds} = -1$ V for IgG modified diamond FET before (solid line) and after exposure to anti IgG (dotted line).

The data in Figs. 2 and 3 show that modification of the diamond surface with IgG yields the ability to selectively recognize and detect the corresponding antibody, anti IgG, in this Bio-FET device. The selective binding of anti IgG, and the *absence* of binding of anti IgM to the IgG-modified diamond surface were further verified using fluorescently labeled antibodies. In the FET device, we attribute the decrease in I_{ds} to positive charges on the anti-IgG molecules, which partially pinch off conduction through the channel when they bind to IgG that is tethered to the diamond surface. This interpretation is further supported by additional measurements (not shown) of the impedance *perpendicular* to the interface. Measurements on *p*-type silicon and *p*-type diamond films both show that when the exposed surfaces are modified with IgG, exposure to anti IgG increases the impedance across the interface; conversely, on IgG-modified *n*-type silicon, exposure to anti IgG *decreases* the impedance across the interface. These measurements, like similar measurements on DNA-modified silicon^{11,12,23} and diamond⁹ surfaces, demonstrate that biological binding events induce a significant field effect in the semiconductor substrate arising from the static charge (positive for anti IgG, negative for DNA) of the molecules binding to the interface. Measurements perpendicular to the interface detection of biological binding events require measurements at ac frequencies where the overall impedance is dominated by the semiconductor space-charge layer.^{9,11,23} In contrast, the FET device described here is a more direct measure of the field effect induced in the diamond space-charge layer.

Based on the observed signal-to-noise ratio, we estimate the sensitivity in these initial experiments to be ~ 7 $\mu g/ml$ of anti IgG. It is likely that the sensitivity could be further improved by optimizing the physical dimensions of the channel. The sensitivity might be further increased by using

charged "labels" that would bind to the anti-IgG molecule and provide it with additional charged groups, in a manner similar to the use of fluorescent labels in the widely used enzyme linked immunosorbent assay ("ELISA").²⁴

While previous work has shown that DNA hybridization can be detected using the field-effect transistors prepared by tethering biomolecules to surfaces of silicon or thin silicon dioxide SiO₂ layers on silicon substrates,^{11,12} in many applications the use of diamond is very advantageous because of the excellent stability over a wide range of chemical and electrochemical conditions.^{5,6} Indeed, recent studies have shown DNA-modified diamond surfaces to be remarkably stable even at temperatures well above room temperature.⁸ This suggests that the use of diamond thin films as the basis for bio-FET devices may be particularly advantageous for biosensing applications requiring extraordinary stability and/or in harsh conditions, such as continuous monitoring and environmental sensing.

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