A charge pumping technique to identify biomolecular charge polarity using a nanogap embedded biotransistor

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Charge pumping technique is investigated to identify biomolecular charge polarity using a nanogap-embedded biotransistor. Biomolecules immobilized in a nanogap provide additional charges in the gate dielectric. They give rise to a change in the charge pumping current, as detected by applying a designed pulse waveform. The measured results are analyzed with the aid of numerical simulations. The proposed charge pumping technique represents an insightful method of investigating the electrical properties of biomolecules beyond biosensing. © *2010 American Institute of Physics*. [doi:10.1063/1.3473819]

Electrical label-free detection of biomolecules is one of the widely researched topics in nanotechnology at present. Various types of detection techniques and nanostructures have been demonstrated to realize an improved biosensor. The authors in a previous work demonstrated a unique biomolecular detection method that was based on charge pumping technique with a nanogap-embedded biotransistor (simply biotransistor).[1,2] In the proposed technique, the trap density of the gate dielectric in a field-effect transistor (FET) is characterized as a sensing parameter. When additional trap states are provided by biomolecules immobilized inside the nanogap carved by the partial etching of the gate dielectric, variation in the trap density results in a measurable change in the charge pumping current (I_{cp}). This change was analyzed quantitatively by charge pumping technique in a highly sensitive and stable manner.[1] In particular, the effects on I_{cp} by intrinsically retained charges in biomolecules have been investigated comprehensively.[2] Moreover, the charge pumping technique has the potential to analyze various aspects of biomolecules electrically. Hence, not only does it enable detection of the biomolecules, it extracts their fundamental electrical properties. The present study focuses primarily on an analysis method to identify the biomolecular charge polarity using the charge pumping technique.

The measurement setup of the charge pumping technique as it analyzes the trap density in the gate dielectric as well as the numerical simulation procedure and details of the biotransistor fabrication process are available in the literature.[1,2] The proposed biotransistor has a partially etched gate dielectric region, i.e., a nanogap region. Immobilized biomolecules in this nanogap region lead to the modulation of I_{cp} according to the intrinsically retained charges in the biomolecules as well as the variation in the trap density in the gate dielectric.[2] When negatively charged biomolecules are immobilized in the nanogaps, the threshold voltage (V_T) of the biotransistor is not uniform along the channel but instead increases locally, as shown in Fig. 1(a). In contrast, when positively charged biomolecules are immobilized in the nanogaps, V_T decreases locally, as shown in Fig. 1(b). It should be noted that I_{cp} can be generated only when the FET is switched between the inversion mode and the accumulation mode.[3,4] Accordingly, if the maximum peak level of the pulse (V_h) for charge pumping is lower than V_T, the channel cannot be switched to the inversion mode. Therefore, I_{cp} cannot be generated from a noninverted channel.

Using this characteristic of charge pumping, the biomolecular charge polarity can be determined through the use of the biotransistor. In the application of the pulse waveform to analyze the retained charge polarity of biomolecules, the minimum peak level of the pulse (V_b) is fixed and V_h is gradually increased, as shown in Figs. 1(a) and 1(b). Figure 1(c) shows the expected I_{cp} characteristics as a function of V_h according to the biomolecular charge polarity. In the case of a fresh biotransistor, the measured value of I_{cp} suddenly in-

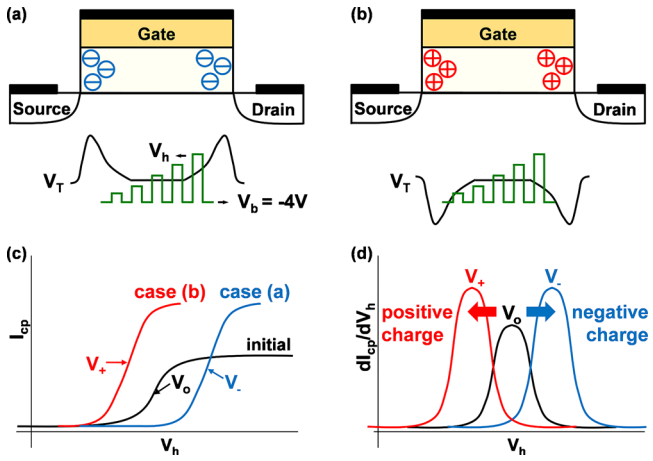

FIG. 1. (Color online) (a) Schematic diagram showing the operational principle of the negatively charged biomolecules. (b) Schematic diagram showing the operational principle of the positively charged biomolecules. (c) The expected I_{cp} values dependent on V_h. (d) The corresponding values of dI_{cp}/dV_h vs V_h.

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creases when V_h exceeds V_T because all of the channel regions are inverted. On the other hand, when charged biomolecules are immobilized in the nanogap, the I_{cp}-V_h characteristic is different from that of a fresh biotransistor. First, the maximum value of I_{cp} is increased due to the extra trap states arising from the biomolecules.[2,5] In addition, when I_{cp} is suddenly changed, the specific voltage to show the peak of dI_{cp}/dV_h (i.e., V_- and V_+) is shifted from V_o due to the locally varied V_T near the nanogap region. It is known that a shift in V_- and V_+ by V_h implies the existence of extra charges in the gate dielectric.[5] Therefore, biomolecular charge polarity can be identified from dI_{cp}/dV_h versus V_h, as shown in Fig. 1(d). When negatively charged biomolecules are immobilized in the nanogaps, V_- will increase more than V_o due to the increased value of V_T near the nanogap region. Similarly, when positively charged biomolecules are immobilized, V_+ will decrease more than V_o due to the decreased V_T near the nanogap region. The aforementioned trend is valid only in a biotransistor based on an n-channel FET. For a biotransistor based on a p-channel FET, the shift in V_- and V_+ would be opposite.

To verify these predictions, a numerical device simulation was carried out.[1,2] In this simulation, all dimensions and device parameters are fitted to the fabricated biotransistor, and additional charges and traps are intentionally assigned in the nanogap region. Figures 2(a) and 2(b) show I_{cp}-V_h curves according to the amount of assigned negative and positive charges, respectively. These simulation results are in good agreement with the abovementioned expectations. When more charges are included in the nanogap region, additional shifts will occur as compared with those occurring in the initial case. The shifts in dI_{cp}/dV_h as a function of V_h are compared in Fig. 2(c). Hence, the biomolecular charge polarity can be distinguished from the peak of dI_{cp}/dV_h.

Experimental verification was carried out through use of the well-known biotin-streptavidin binding method. The immobilization of biotin on the nanogap surface was prepared by a two-step procedure. The biotransistors were washed with an ethanol solution to remove contaminants and were then immersed in a 1% (3-aminopropyl)triethoxysilane (APTES) ethanol solution for 30 min. Subsequently, they were washed with pure ethanol and heated at 120 °C for 20 min to remove surplus ethanol. Finally, sulfo-NHS-LC-biotin (10 mM) in phosphate-buffered saline (PBS) was used in a reaction with the APTES-modified surface for 1 h. The unreacted sulfo-NHS-LC-biotin was removed by deionized water (DW). Without a time delay, the biotinylated device was immersed into a streptavidin/PBS solution for another hour. Excess streptavidin solution was washed away with PBS and DW, and the device dried in a stream of dry N_2 gas. It is known that this APTES has positive charges in a solution at a neutral pH.[6,7] As all bioreagent solutions were prepared and adjusted to pH 7.4 using PBS, APTES was positively charged as used in the present experiment. In contrast, biotin (pI=3.5) and streptavidin (pI=5–6) were negative as their pI values that were lower than that of PBS.[8,9]

Based on the biomolecular charge polarity, Figs. 3(a)–3(c) show the measured I_{cp}-V_h characteristics after each bioexperiment step. When the nanogap surface was modified by APTES, the I_{cp} curve shifted slightly to the left [Fig. 3(a)]. This was due to the decreased channel V_T caused by the positively charged APTES in which the channel is inverted earlier compared to when a fresh biotransistor is used. Consequently, a sudden change in the value of I_{cp} occurs at the lowered voltage. It should be noted that the maximum value of I_{cp} does not change in this case. Hence, it reveals that APTES did not provide extra trap states inside the gate dielectric. It was reported that APTES showed a good insu-

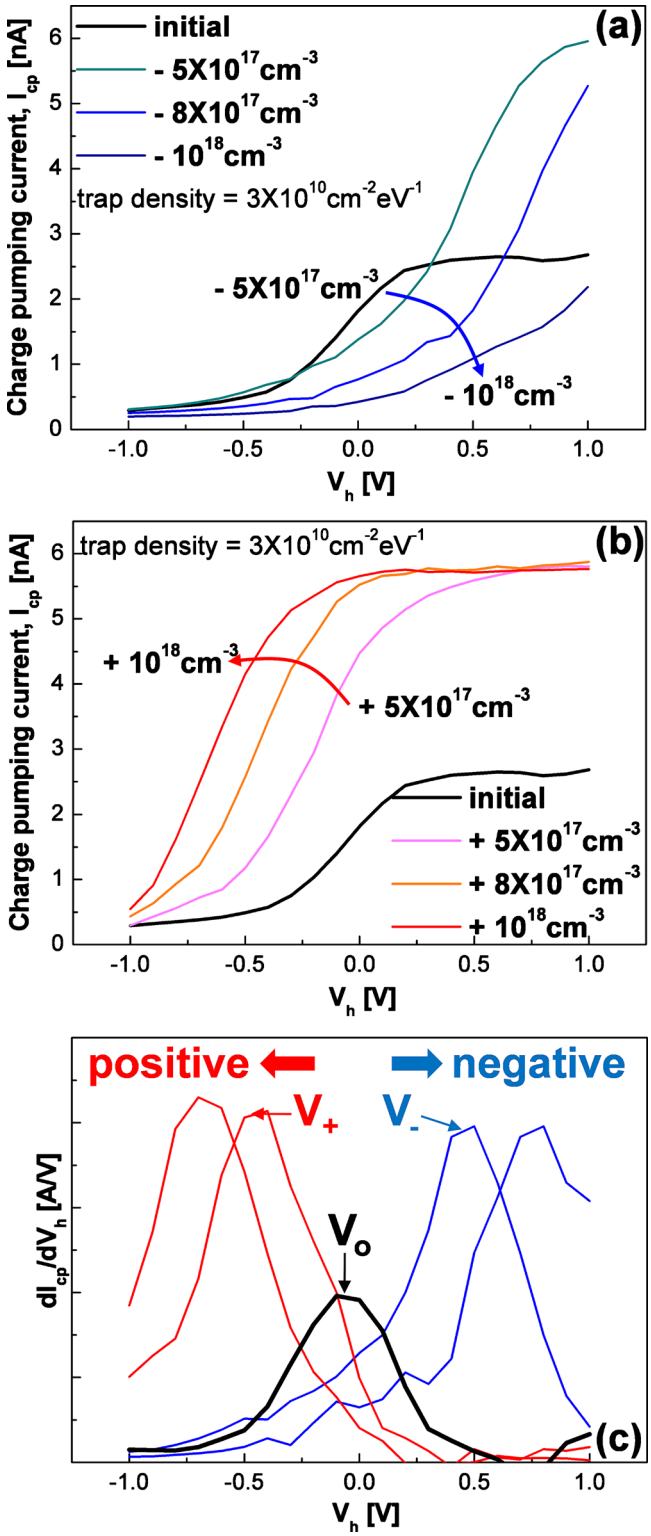

FIG. 2. (Color online) Numerical simulation results: (a) The calculated I_{cp} values vs V_h varied when negative charges are assigned in the nanogap region. (b) The calculated I_{cp} values vs V_h varied when positive charges are assigned in the nanogap region. (c) The extracted behavior of dI_{cp}/dV_h vs V_h. The biomolecular charge polarity is distinguishable from the shift direction from the plot of dI_{cp}/dV_h vs V_h.

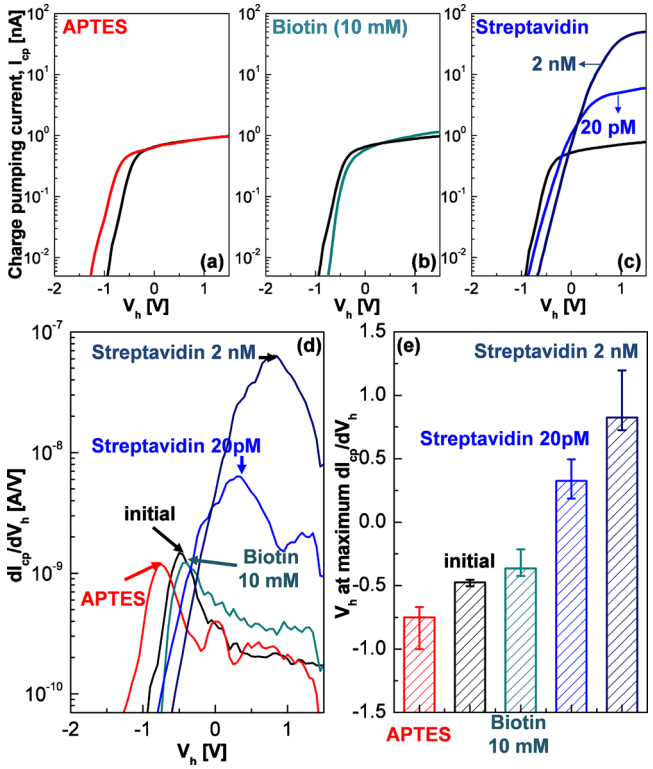

FIG. 3. (Color online) Experimental results: (a) The measured values of I_{cp} vs V_h when the nanogaps are modified by positively charged APTES. (b) The measured values of I_{cp} vs V_h when negatively charged biotin is immobilized in the nanogaps. (c) The measured I_{cp} vs V_h values when they vary depending on the (negatively charged) streptavidin concentration. (d) The dI_{cp}/dV_h vs V_h characteristics. The shift direction in the $dI_{cp}/dV_h - V_h$ plot indicates the charge polarity of the biomolecules. (e) The V_h value at maximum dI_{cp}/dV_h. Each instance of error-bar data was extracted from 20 randomly selected different devices.

lating property, which is appropriate for a gate dielectric in a FET.[10,11] Thus, it can be inferred that the shift in the curve is primarily due to the positive charge of APTES. After biotin immobilization, the I_{cp} curve was shifted to the right [Fig. 3(b)] due to the increased channel V_T stemming from the negatively charged biotin. Finally, after the specific binding of biotin-streptavidin, the I_{cp} curve was shifted further to the right. This shift depended on the concentration of the streptavidin [Fig. 3(c)]. The maximum value of I_{cp} was also changed significantly due to the extra trap states provided by the streptavidin. These modulations of the maximum value of I_{cp} are consistent with previous work by the authors.[2] Consequently, the biomolecular charge polarity is distinguishable from the shift direction in the I_{cp}-V_h plots as compared to the case of a fresh biotransistor. Figure 3(d) clearly shows the behavior of the plot of the dI_{cp}/dV_h versus the V_h curves. In addition, Fig. 3(e) shows the V_h value at maximum dI_{cp}/dV_h (i.e., V_o, V_-, and V_+). Each instance of error-bar data was extracted from 20 randomly selected different devices. It can be clearly shown that the identification of charge polarity by charge pumping has a good reproducibility and reliability. However, these data cannot make sure that the charge polarity of biomolecules is determined according to the pH value of buffer solution even in dry condition. The characteristics of V_- and V_+ shift started to be disappeared after 2 h past (data are not shown). We speculate that buffer solution inside nanogap region is not completely dried up by N_2 stream, and this solution is maintained for approximately 2 h. Because all measurements were carried out within 1 h, consequently, the charge polarity expected by pI value can be identified by the charge pumping method properly.

In summary, a charge pumping technique was investigated to identify the biomolecular charge polarity using a nanogap-embedded biotransistor. By applying the designed pulse waveform for charge pumping, the biomolecular charge polarity was determined by the shift in the direction in dI_{cp}/dV_h. The proposed charge pumping technique shows potential in that various electrical aspects of the biomolecules can be analyzed as an investigation tool to extract their fundamental properties and their biosensing characteristics.

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