An AFM-FET Biosensor for Proteomic Screening

Byung I. Kim, Joseph O. Holmes, Moon S. Ryu, and P. Deschateletes

Abstract—**An atomic force microscope (AFM) and a field effect transistor (FET) were integrated into one functioning novel biosensor to probe single molecular interactions between biomolecules, such as proteins and ligands. The interactions between avidin-biotin complexes were studied using the AFM-FET biosensor. Biotin molecules were attached to the AFM cantilever via flexible polyethylene glycol cross-linkers, and avidin molecules were bound to the palladium gate surface of the FET via the cross-linker molecules, cystamine and glutaraldehyde. The force of the AFM and the FET current between the drain and source was measured as a function of distance when the biotin molecules approached and retracted from the avidin molecules. The measurement data showed that the FET current changed abruptly when binding and unbinding events occurred between the two molecules. This change in the current was generated from the corresponding electric potential, which was related to the magnitude of unbinding force during the binding and unbinding events. The relationship suggests that the AFM-FET biosensor can provide potential voltages generated during the biding and unbinding events. This additional information on molecular interactions at the single molecular level could potentially be used as a quantitative and efficient method for detecting single molecular interactions. The novel AFM-FET biosensor is potentially advantageous over current drug screening methods based on microfluidics, due to the AFM-FET ability to record single interactions more quickly and accurately.**

Index Terms—**AFM, Biosensor, FET, Molecular interactions**

I. Introduction

The majority of biological functions are carried out at the molecular level by interactions between various biomolecules. Because these interactions are often the target of pharmaceutical agents, drug screening techniques must be capable of sensing the interactions. The most popular drug screening method is microfluidics, which utilizes tiny fluid channels that sense these various biomolecular interactions [1], [2]; however, this method has some critical limitations including difficulty in fluid flow due to the hydrodynamics especially at the boundaries of the microfluidic channels. This can produce discrepancies between the predicted and actual behavior of how the fluids mix, flow, and operate. Also, when microfluidics is applied to proteomic screening, sensors are used to detect these interactions between biomolecules. The saturation of the sensor impedes the throughput capacity.

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Field-effect transistors (FET) have been widely used as biosensors, but are generally used with a large number of molecules to obtain a sufficient signal [3]. The measurements taken with the FET-based biosensors are mostly "on" or "off" measurements, which are determined by the presence or absence of a reaction. An ion-sensitive FET was described by Bergveld in the early 1970s and set the stage for the development of many generations of chemical and biological sensors [4], [5]. A specific type of FET, the p-type metal oxide semiconductor FET (pMOSFET) appears to be a promising biosensor device. The pMOSFET contains holes in the channel, also known as the inversion layer, opposite in carrier type to the substrate. An atomic force microscope (AFM) has shown the ability to measure molecular interactions down to the single molecular level and to control the distance between a ligand and a receptor protein up to subatomic resolution.

Incorporation of the AFM into FET has not been previously reported to our knowledge. The integration of AFM and FET technologies has the potential to provide not only valuable information about these biomolecular interactions that has not been accomplished by other methods, but also a much more rapid drug screening technique. The ability to control single molecules will allow for the comprehensive study of biomolecular interactions at the single-molecular level.

II. Materials and Methods

The AFM-FET biosensor combines two separate pieces of technology into a single functioning sensor. The AutoProbe LS AFM system (obtained from former Park Scientific Instruments) was employed to manipulate single molecules and to measure unbinding forces of biological receptor-ligand systems, while the FET (obtained from the Rochester Institute of Technology, Rochester, NY) was used for chemical and biological sensing. The FET has a layered structure consisting of the source, gate and drain [6]. The gate is located between the source and drain and is where biomolecules can be attached. A highly sensitive enhancement mode pMOSFET was chosen to reduce the background noise signal compared to "depletion mode" FET. The FET sensor was mounted to the AFM stage on the AFM piezo scanner.

A SiAl wire with diameter of 25 μm was used for wire-bonding the FET to connect external wire lead lines to the measurement electronics. One of the most troublesome problems was the current leaking into the buffer solution, *phosphate buffered saline* (PBS). Insulating the wires with UV epoxy solved this problem. A new system for the measurement of the FET current at the AFM stage was

designed and constructed. The high-voltage signal controller SPM100 (purchased from RHK Technology, Troy, MI) was used to change the tip-sample distance in the z direction by regulating the high-voltage sent to the piezo tube. Fig 1(a) displays the combined system for the FET current and AFM force measurements. Additionally, the RHK controls the voltage sent to the FET by applying a voltage to the FET source, monitoring the FET drain source in current, by applying a bias voltage to the FET gate (see Fig. 1(b)). The minuscule size of the FET chip (seen resting against the sharpened tip of a conventional #2 pencil in Fig. 1(c)) adds minimal bulk to the piezo scanner and therefore avoids any additional scanning interference.

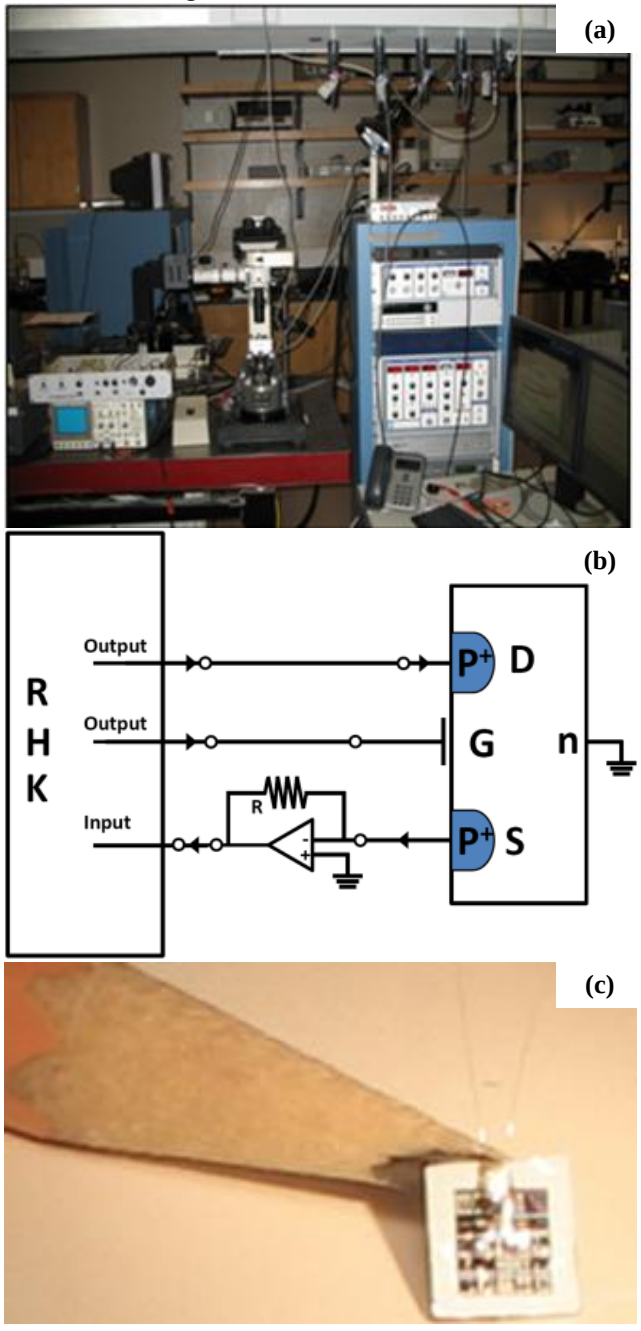


Fig. 1. (a) From left to right: oscilloscope/custom-built setup for FET current measurement, AFM-FET, RHK controller and computer, (b) Basic circuit diagram for FET family of curves measurement, (c) Close up of only the FET chip with lead wires attached.

In the AFM, the interaction force was recorded as a function of distance between two biomolecules using an optical beam detection system (as shown being made up of a laser and a position-sensitive photodiode as in Fig. 2(a)). The distance was controlled using a piezo tube whose extension is determined by a computer controlled high voltage [7-9].

In the AFM-FET biosensor, biotin and avidin molecules were attached to the AFM cantilever and to the FET gate surface, respectively. Bioconjugate techniques were employed to tether the biomolecules (a ligand of choice, biotin, and its specific counterpart biomolecule, avidin) to the AFM cantilever and to the gate surface (see Fig. 2(a) and 2(b)).

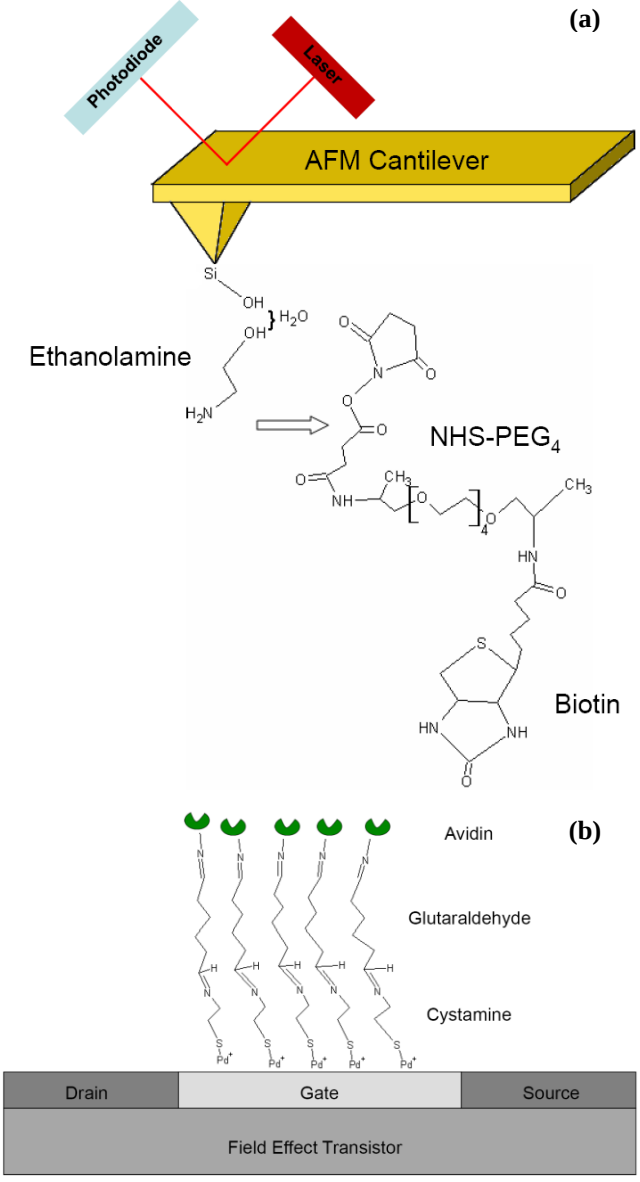


Fig. 2. (a) Schematic representation of a biotin molecule to the cantilever along with an optical beam deflection detection scheme [10]. Biotin-PEG-NHS is tethered to the AFM tip using an amide bond formation. (b) The structure of FET with the gate tethered with the avidin through a spacer molecule, glutaraldehyde.

The biotin molecules were tethered to the silicon nitride surface of the AFM cantilever via flexible polyethylene glycol (PEG) molecules (as seen in Fig. 2(a)) [10]. Biotin molecules were attached to the tip of the AFM cantilever in two steps: 1) esterification with ethanolamine and 2) functionalization with the tethering system called biotin-PEG-NHS.

First, 6.6 g of ethanolamine HCl (obtained from Fisher Scientific, Pittsburgh, PA) was dissolved in 12 mL of

dimethyl sulfoxide (DMSO) with molecular sieve beads. Next, the probes were immersed in the solution overnight, rinsed with DMSO and ethanol three times each, and dried under a gentle stream of nitrogen. After that, the probes were placed in a 1 mg/mL chloroform solution of biotin-PEG-NHS (obtained from Thermo Scientific, Waltham, MA) and tri-ethylamine for three hours. The probes were then rinsed in chloroform, air dried, and rinsed with PBS.

The avidin was applied to the palladium surface of the chip for the use of thiol-linkage. Then, the avidin coated surface was emersed in a 1 mg/mL chloroform solution of avidin horseradish peroxidase (obtained from eBioscience, San Diego, CA) and tri-ethylamine for three hours. The thiol-linkage allowed the formation of a highly ordered self-assembled monolayer of cystamine molecules (cystamine dihydrochloride molecules obtained from Fisher Scientific, Pittsburgh, PA) on the palladium FET gate surface (seen in Fig. 2(b)). Avidin proteins then were tethered to the cystamine via the amine-reactive homobifunctional cross-linker glutaraldehyde (obtained from Sigma-Aldrich, St. Louis, MO). The glutaraldehyde is a relatively short linker, approximately 0.75 nm, for better transduction of the binding and unbinding events without losing bioactivity of the protein avidin molecules. Both spacer molecules, PEG and glutaraldehyde/cystamine cross-linkers, were employed to reduce steric hindrance between the biotin and the avidin, allowing for optimal binding.

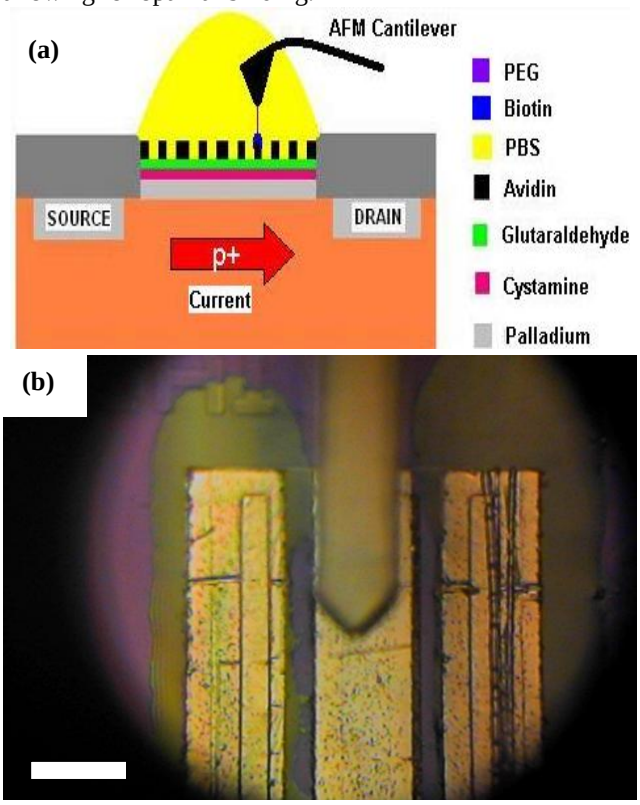


Fig. 3. Integration of AFM and FET into one biosensor (schematic drawing (a) and real picture of actual AFM-FET biosensor (b)). The scale bar represents a length of 50 μm. The cantilever is shown in the middle upper part in Fig (b) and is the unit of force measurement as well as biomolecular manipulation with respect to the surface. Both drain (left) and source (right) electrodes are covered with UV epoxy to prevent both electrode materials from electrochemically reacting with electrolyte PBS solution.

The FET and the AFM were integrated into one biosensor, as shown in the diagram, Fig. 3(a). The cantilever has an attached PEG linker with a biotin end group that will interact with the avidin layer on the FET chip. The diagram displays a current running from the source to the drain in a FET chip, layered from bottom to top with palladium, cystamine, glutaraldeyhde, avidin, and covered with the PBS solution. A picture of the integrated AFM-FET biosensor taken by optical microscope is seen in Fig. 3(b). The beam-shaped object located in the upper midsection of the picture corresponds to the AFM cantilever. The biomolecules were attached below the cantilever. The three bands shown below the cantilever are part of the FET, the source, gate, and drain from left to right respectively. The source (positive) terminal and the drain (negative) terminal are used to apply a voltage across the device. Below the gate surface the conduction channel exists and is controlled by biasing the gate. The gate bias creates an electric field that opens or closes the conduction band below its surface. The FET has a conduction channel with positive charges (doped with phosphorous atoms to allow for a conduction band) to carry current from the source to the drain [11]. Each tested FET was checked by measuring the current versus the voltage in air. Once a working FET was identified, the avidin-biotin receptor system was implanted into the FET sensor.

The cantilever with biotin was brought into contact with the gate of the FET with avidin. Then, the cantilever was retracted from the gate surface. The AFM force associated with their interaction was recorded as a function of distance between the two biomolecules. The FET current was also simultaneously recorded as a function of distance while the cantilever approached and retracted from the surface. The AFM force-distance curve was compared with the FET current-distance curve. The binding and unbinding affinity of the receptor-ligand system was judged via change of the FET current and unbinding force. The voltage across the drain and source was held constant (V_{DS}= -2.0V) while the avidin-biotin system was pulled apart in PBS (pH of 7.4).

III. Results and Discussion

The family curves were generated by measuring the FET current across the channel between the drain and source as a function of the gate-source voltage in Fig. 4(a). The calibration curve of the FET sensor was used to calculate the potential to generate the current created during the binding and unbinding events. When the saturated drain current was plotted as a function of the gate-source voltage (seen in Fig 4(b)), the drain current decreased in a parabolic fashion with the gate-source voltage. The parabolic curve was quantified by the following equation [6]:

$$I_D = k(V_{GS} - V_{GS,th})^2 \quad (1)$$

where I_D is drain current, k is the construction parameter, V_{GS} is the gate voltage, and $V_{GS,th}$ is the threshold voltage. The k and $V_{GS,th}$ were found to be 47.38 μA/V^2 and -0.44 V, respectively, when Fig. 4(b) was fitted (seen by the blue line) using the equation. The (1) along with the fitted constants were used as a reference relationship between the I_D and V_{GS} for the interpretation of observed current changes caused by binding and unbinding events.

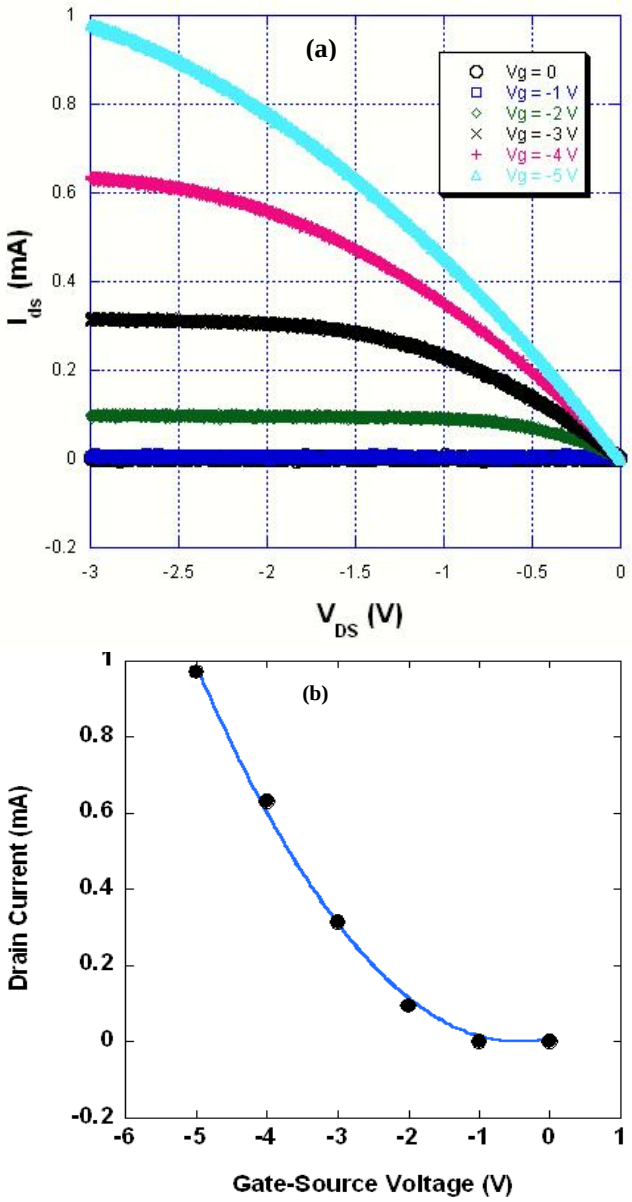
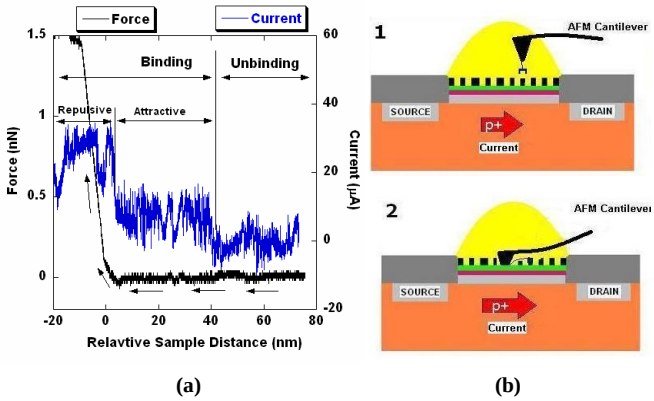


Fig. 4. (a) The FET family curve. The drain current increases as the bias voltage increases. (b) The drain current (I_D) as a function of the gate-source voltage, V_{GS}. The line represents a curve fitting with a hyperbolic equation.

To observe how the FET current changes during the biomolecular binding process, simultaneous current and force curve measurements were taken during the tip approach (seen in Fig 5(a)).The FET current with 10 μA was turned on at 40 nm distance between the tip and the sample when the biotin functionalized tip approached the avidin functionalized gate surface of the FET. A second jump around 16 μA in the FET current appeared near the sample surface or at 0 nm as the tip approached further. Considering that the total length of spacer molecules is between 40 nm - 50 nm, the first current jump 10 μA is assigned to be the binding event between attached biotin and avidin molecules at the top layers on both the tip and the surface, respectively. The current can be correlated with the binding event between an avidin molecule and a biotin molecule.

This current change is remarkable because the AFM force curve did not show any notable change of force at the binding point (43 nm) compared to the background signal. The single binding event between two biomolecules is generally not detectable in the force curve during tip-approach. The current jump of FET is a new way to probe the binding event between two biomolecules during the tip-approach. The change of FET current occurs beneath the gate when a binding event occurs. As a certain gate potential voltage typically causes the observed current jump, this voltage is expected to originate from the binding process between the two molecules. At this point, the creation mechanism of potential voltage is not well understood; however, the binding event should create enough of an electric field to turn on the current between the drain and source. The second current jump is assigned to the touching of the tip-end to the surface molecular layers because it occurs just above the surface.

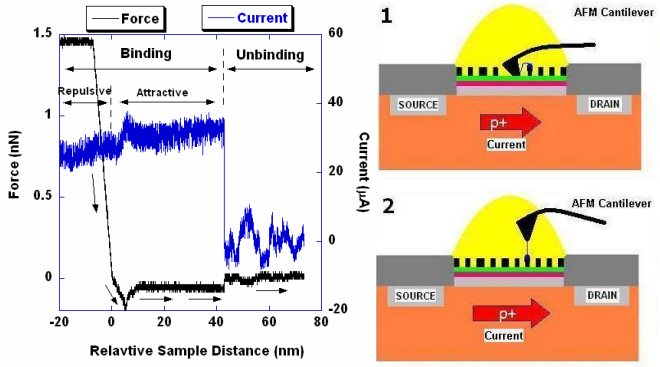


(a) (b)

Fig. 5. (a) Simultaneous measurement of current and force curves during the tip approach. The arrows below the force curve represent the direction of the tip towards the surface. The approach current and force curves showed a 16 μA current change around 40 nm thought to be a specific binding event between avidin and biotin. (b) Two schematic drawings, (1) and (2), are given on the right side showing the relation between FET current and the position of the tip. Drawing (1) represents the unbinded state between the biotin and the avidin in which the FET current is not turned on. Drawing (2) represents a binding state between the biotin and the avidin, turning on the FET. The arrow represents the direction and magnitude of the FET current.

This single molecular binding event and the tip-touching event during the approach are described with two schematic drawings (seen in Fig. 5(b)). The avidin-biotin binding configurations were conjectured from approach and retraction force-curves. Based on this information, the current changes were correlated with the binding states during the tip-excursion. As the tip approaches (Drawing (1)), the binding event between avidin and biotin causes the sudden jump in the FET sensor. After the biomolecular binding, the interaction force becomes repulsive due to the tip-interaction with glutaraldehyde (green layer in Drawing (2)), causing the nonspecific interaction in the force curve.

Fig 6(a) shows simultaneous current and force curves during the tip retraction. This single molecular unbinding event during the retraction is described with two schematic drawings (seen in Fig. 6(b)). As the tip retracts (Drawing (1)), the interaction force that had become repulsive during approach due to the tip-interaction with glutaraldehyde reverts back to an attractive interaction force as the tip separates from the glutaraldehyde. Once the tip reaches the full stretch-distance of the spacer molecule (Drawing (2)), the tension builds and the unbinding event occurs as intermolecular forces are overcome by the mechanical force of the cantilever being retracted.



(a) (b)

Fig. 6. (a) Simultaneous measurement of current and force curves during the tip retraction. The arrows below the force curve represent the direction of the tip towards the surface.

Retraction current and force curves showed a 33 μA current change around 43 nm thought to be a specific unbinding event between avidin and biotin. (b) Two schematic drawings are given on the right side showing the relation between the FET current and the position of the tip. Drawing (1) represents a binding state between the biotin and the avidin, turning on the FET. Drawing (2) represents a state of high tension during the unbinding process right before separation occurs. The arrow represents the direction and magnitude of the FET current.

The stretched length of the spacer molecule is found to be around 40-50 nm from the force curve on retraction (seen in Fig. 7. A nonspecific attractive force with 200 pN is developed at the distance 4 nm in Fig. 7 due to the PEG, glutalydehyde, and cystamine layers. Although the PEG stretching behavior is not well-resolved in detail due to the high spring constant of the cantilever, the force decrease in the range between 10 nm – 40 nm in Fig. 7 clearly indicates the stretching behavior of spacer polymers (PEG linker and glutaraldehyde) during the unbinding process. In addition to the stretching polymer length (43 nm), the jump of unbinding force around 60 pN is consistent with those literature values [12].

The current curve that was taken simultaneously with the force curve also shows an abrupt jump-down of the FET current from 35 μA to zero at the distance 43 nm (as seen in Fig. 7). The current change in Fig. 7 is due to the unbinding event between the avidin and the biotin molecules, because the force curve also has a jump at 43 nm. This result indicates that the current change is not reversible during the binding and unbinding process. The mechanism of this irreversibility in the FET current observed during the binding and unbinding events is not clear at this point. One possible explanation of this irreversibility may be related to the change of activation energy barriers during the tip excursion between binding state and unbinding state, if we assume the FET current is dependent on the binding affinity. The avidin-biotin complex may have stronger binding with time during the excursion, which influenced the measured FET current. This hypothesis needs to be explored for future quantitative proteomic databases that will utilize the AFM-FET hybrid technology. Interestingly, the FET current change is 3-4 μA during the nonspecific interaction at a distance of 4 nm, which is an order of magnitude smaller than the current change during unbinding event 30-40 μA, although the nonspecific force is more than three times bigger than the specific force.

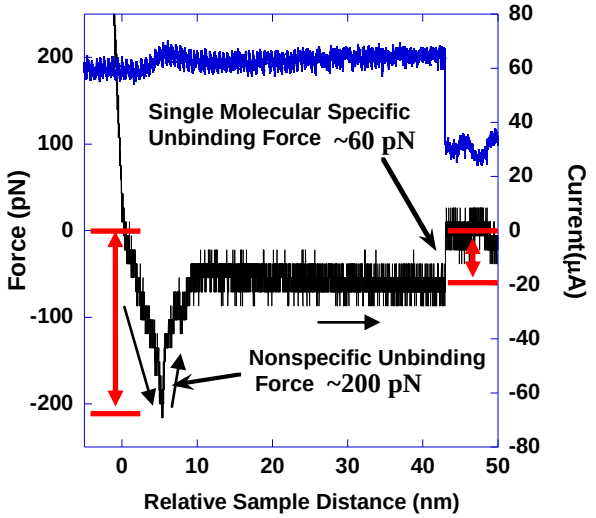


Fig. 7. Zoomed current and force versus distance curves during the tip retraction.

A potential voltage across the gate and the source was calculated durring the binding and unbinding events between the biomolecules biotin and avidin. The calculation provides some rough idea to how the binding generates the amount of current observed in the approach (Fig. 5) and retraction (Fig. 6) measurements. Using Fig. 5 and Fig. 6, I_D was found to be 16 μA and 33 μA for binding and unbinding events, respectively. Therefore, V_{GS} is calculated to be -1.02 V and -1.27 V for approach (during binding) and retraction (during unbinding), respectively, using (1) and the calibrated constants, k and $V_{GS,th}$. Because these two V_{GS} values are larger than $V_{GS,th}$ and V_{DS} is larger than V_{GS}, the FET sensor was operated in the saturation mode when it detected the binding and unbinding events. Again, the magnitude of the unbinding potential voltage is larger than that of the binding potential voltage. The binding and unbinding potential voltages provides a more comprehensive way of documenting interactions at the single molecular level by providing additional information on molecular interactions at the single molecular level. For example, the contribution of the nonspecific interactions to the FET current is much smaller than the specific interactions although the nonspecific adhesive force larger than the binding and unbinding forces. Specific interactions, such as enzyme reactions, generally involve more charge creation or change of chemical bonding configurations than that of nonspecific interactions, such as physisorption. The integrated AFM-FET biosensor extends the current limitations of the FET biosensors in measuring the change in FET current during single biomolecular interactions.

The ability of the AFM-FET biosensor to record specific values from single molecular interactions is advantageous over currently used microfluidics because microfluidics is not able to measure these interactions as quickly or as accurately. Additionally, the AFM-FET biosensor does not experience hydrodynamic problems commonly associated with microfluidics unless approaching and retracting at far higher speeds than needed for molecular interaction detection. These high speeds are not necessary as the AFM-FET biosensor is able to measure force and current changes simultaneously

during tip excursion, whereas microfluidics requires sending the molecule down a channel for analysis after binding and unbinding events. The AFM-FET is also able to take repetitive measurements during proteomic screening, which microfluidics is incapable of doing because the throughput capacity is impeded by the saturation of the biosensor. The AFM-FET based biosensor will be an important step towards the development of a novel biosensor for proteomic screening.

IV. Conclusion

The AFM and FET were integrated into one functioning biosensor and its efficiency at detecting single molecular binding and unbinding events was exhibited by probing the interactions between avidin-biotin complexes. Biotin molecules were attached to the AFM cantilever via flexible PEG cross-linkers, and avidin molecules were bound to the palladium gate surface of the FET via the cross-linker molecules of cystamine and glutaraldehyde. The force was measured as a function of distance for the AFM and the FET current between the drain and source when the biotin molecules approached and retracted from the avidin molecules. Abrupt changes in the FET current were observed when binding and unbinding events occurred between the two molecules. The magnitude of the binding and unbinding forces was related to the change in the current generated from the corresponding electric potential. The simultaneous measurement of force and current is important to understand molecular interactions at the single molecular level comprehensively. This result indicates that the introduced AFM-FET biosensor can potentially be used for future proteomic screening through efficient detection of single molecular interactions and the collection of quantitative data.

Acknowledgment

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