

NICKEL CHLORIDE-MEDIATED PROTEIN ATTACHMENT TO MOLYBDENUM DISULFIDE FOR BIOSENSING APPLICATIONS

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Abstract

Single- and few-layer molybdenum disulfide (MoS_2) thin films, which have recently been synthesized for the first time, are of great interest for potential applications due to their two-dimensional structure and electronic properties. With a bandgap of 1.8 eV, conduction through this material can be tuned between on and off states, a property that graphene, a more studied two dimensional material, does not possess. Furthermore MoS_2 presents high thermal and chemical stability, which allows the creation of high-performance nano-electric devices such as field effect transistors (FETs), which could be used as ultrasensitive sensors for clinically-relevant proteins and other biomolecules. These kinds of sensors are currently fabricated with carbon based materials such as nanotubes and graphene and have detection limits at the pico-molar levels. However, the bandgap of MoS_2 could allow for lower concentration detections. This study investigates the process of nickel chloride (NiCl_2) mediated protein attachment to exfoliated MoS_2 flakes using different methods of purification and different concentrations of NiCl_2 . Testing our mechanism is ongoing and will specify the most beneficial conditions of NiCl_2 needed to attach the highest density of proteins to the MoS_2 . This is the first step towards building a biosensor based upon molybdenum disulfide.

Index Terms — Molybdenum disulfide, protein attachment, nickel chloride

I. INTRODUCTION

Graphene and carbon nanotubes are materials currently being studied for sensing applications because their low dimensionality means every atom is on the surface exposed to the environment. Molecular biomarkers of certain types of cancer have been detected at the pg/mL level using functionalized graphene field effect transistors [1], a level approximately 1000 times more sensitive than current clinical technologies. Because of the sensitivity and selectivity that these types of sensors offer, these materials are being heavily investigated.

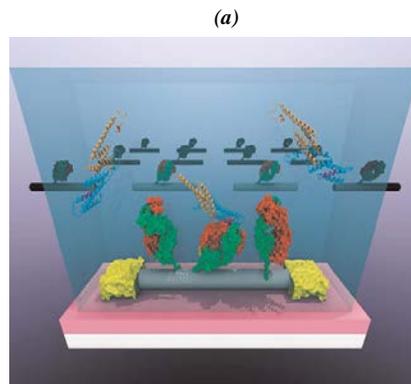
One potential problem for some applications is that graphene does not have a bandgap. This means that the field effect transistor may approach minimum levels of current but it can never be turned off. This issue may be resolved through the use of next-generation monolayer materials, such as molybdenum disulfide (MoS_2) [2], which is a direct gap semiconductor with a band gap of 1.8 eV [3].

The purpose of this project is to develop a method to attach proteins to monolayers of molybdenum disulfide in order to create a sensor that detects biomarkers of diseases. These devices should be able to determine the early stages of several anomalies by detecting antigen molecules bound to sensor and changing the electrostatic environment of the sensor.

II. BACKGROUND

A. Biosensors

Biosensors are devices that recognize biological activity through the identification of electric signals detected by transducers. Biosensors are classified by the recognition element or the transduction process. [4]



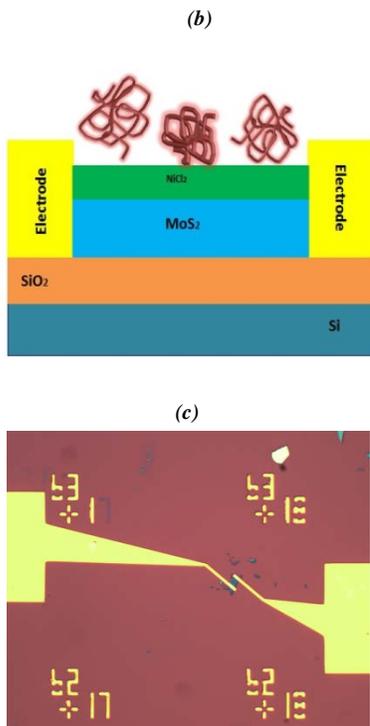


Fig. 1. (a) Carbon nanotube field-effect transistor biosensor used in the detection of prostate cancer biomarkers. [1] (b) Schematic of proposed molybdenum disulfide based biosensor. (c) Optical image of molybdenum disulfide sensing device. Electrodes are attached to the flake in order to measure electrostatic changes.

There are several kinds of biosensors, including electrochemical, optical, calorimetric and acoustic sensors. The types of sensors to be synthesized in this investigation are electrochemical devices. Within this category, there are several transduction technologies approached, such as ion-selective electrodes (ISEs), gas-sensing electrodes and field-effect transistors (FETs) [5, 6].

Research nowadays has focused in the capabilities of field-effect transistors. Several methods include the use of 1-dimensional and 2-dimensional materials such as carbon nanotubes and graphene respectively. The benefits of using these biosensors include a high sensitivity compared to the approaches found in clinical standards and a comparable height platform to several biomolecules, providing a compatible area for binding [7]. Therefore, the motivation of the project is to seek further sensitivity enhancements in biosensors using a semiconductor such as molybdenum disulfide.

Nowadays a lot of diseases are detected by methods such as the enzyme-linked immunosorbent assay (ELISA), a test that is based on color change for antibody detection. However, the use of FETs could provide faster, inexpensive results that don't require pure samples and provide much more accurate results [8]. A lot of research has been done in the field of carbon nanotubes FETs. Fig. 1a presents the structure of a

carbon nanotube field effect transistor (CN-FET) as a biosensor for prostate cancer. In the image, the array of nanotubes binds to the protein and produces a change in the current-voltage characterization of the device.

A similar approach is followed in this investigation. On the other hand, instead of carbon nanotubes, flakes of molybdenum disulfide are used. The final sketch of the complete device to be employed as a biosensor is shown in Fig. 1b. In this case, the MoS_2 is bound to nickel chloride and to the receptor protein. These are connected to electrodes that will measure the electrostatic changes when the antigen binds to the sensor. Fig. 1c shows the optical image of a complete device. The image presents electrodes that are attached to the molybdenum disulfide flake. By applying a gate voltage to the system, the source and drain electrodes could measure the electric changes in the system created by the presence of antigens, providing a fast and accurate prognosis of diseases.

B. Molybdenum disulfide

Molybdenum disulfide (MoS_2) is a 3-layer semiconductor formed by sulfur-molybdenum-sulfur bonds. Fig. 2a displays the atomic structure of molybdenum disulfide. Two hexagonal planes describe the structure of the molecule. Each metal atom covalently bonds to a sulfur atom forming a trigonal prism layout [9]. Every molybdenum atom bonds to six sulfur atoms as each sulfur bonds to three molybdenum atoms creating bonds of approximately 1.54 \AA of length and a whole layer of compound of 0.7 nm of thickness. While strong covalent bonds are found in plane, weak Van der Waals forces lie in between the layers of the bulk material.

The electric behavior of molybdenum disulfide depends on the voltage applied to the system. Fig. 2b explains the increasing linear correlation between the drain-source voltage difference and the current of MoS_2 . Literature explains that molybdenum disulfide reaches saturation. However, current investigations are conducting reproducibility experiments to verify this finding. On the other hand as Fig. 2c shows, an exponential increment of current is seen as the gate voltage increases when the drain-source voltage is kept constant [11].

Single-layer flakes of molybdenum disulfide present a bandgap of 1.8 eV . Depending on the number of layers of the compound, this property may decrease by 30 percent reaching values of 1.29 eV [9,10]. A bandgap is the difference of energy between the minimum of the conduction band and maximum of the valence band of a material. There are no allowed electron states in the gap between the two bands [12].

For undoped MoS_2 the valence band is completely full and the conduction band is completely empty. However, the Fermi level, which specifies the highest energy state that is filled, can be altered by changing the electrostatic environment. Electron (hole) carriers can be created in the channel by applying a perpendicular electric field, which is accomplished using a gate voltage in a three terminal field effect transistor

configuration. In practice, the presence of the substrate preferentially inhibits hole conduction, resulting in n-type (electron dominated) transport, as shown in Fig. 2c.

In addition to the presence of a bandgap, MoS₂ presents high thermal stability due to the absence of dangling bonds. This property makes the material optimal for the fabrication of transistors with a low degree of power dissipation, being ideal for nano-electronic and photo-electronic applications. High performance low-power devices for switching applications can be developed with molybdenum disulfide [13, 14].

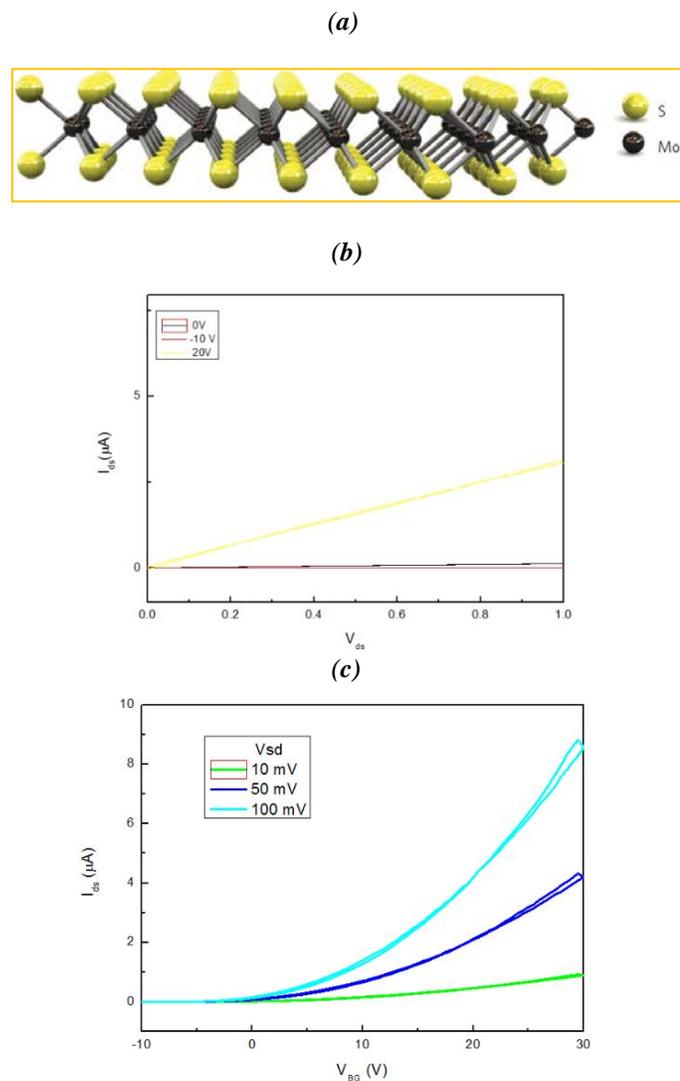


Fig. 2. (a) Structure of bonds between the molybdenum and the sulfur atoms. The S-Mo bond is 1.54 Å. [14] (b) Current vs Source-Drain voltage characteristics seen in MoS₂. (c) Transport properties in a monolayer of molybdenum disulfide, showing strong p-type conduction.

C. Proteins

Proteins are large, highly-specific biomolecules made up of amino acids. Each amino acid is joined together by peptide bonds between its carboxyl and α -amino group [15] as seen in Fig. 3a.

Proteins are found in living organisms and are fundamental for survival. From maintaining the structure of genes to performing reactions in an organism, proteins are a central component of human beings. Proteins including enzymes, cell receptors, and catalysts, perform functions vital to life.

Antigens, cellular receptors and antibodies can be used as biomarkers. Biomarkers are molecules that act as indicators of specific biological conditions. In this investigation, antigens and cellular receptors are the biomarkers targeted because of the immunological response they produce in the body.

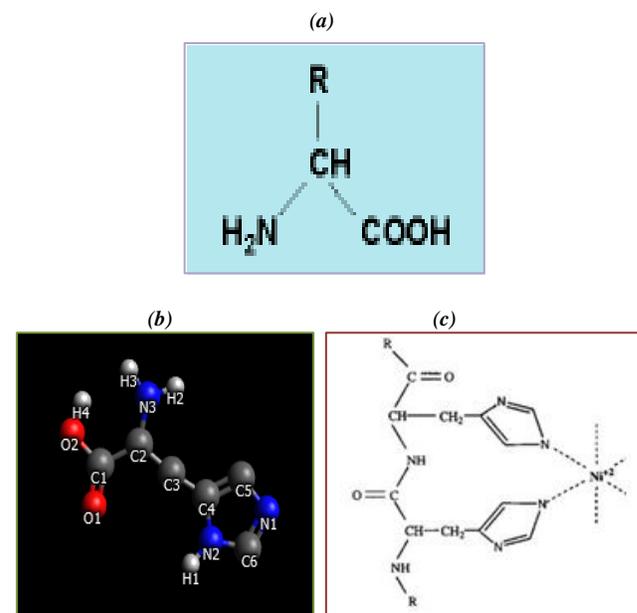


Fig. 3. (a) Structure of an amino acid. The alpha carbon is covalently bonded to the amino group (-NH₂) and to the carboxyl group (-COOH). The -R group represents a heavier molecule that defines the type of amino acid [16]. (b) Structure of a histidine amino acid. The ring characterizes the imidazole group. (c) Structure of a histidine tag bound to nickel through coordinate bonds.

Immobilization of proteins in a biosensor is necessary to analyze the sensitivity of the device. There are several techniques used to immobilize a protein. These include alkylation, peptide bond formation and diazotization. In order to immobilize the proteins in this study, the diazotization treatment was performed using polyhistidine tags found in proteins. [17]

A polyhistidine tag or 6xHis-tag is a combination of at least six histidines found at the –N or –C ends of the protein [18]. Its formula is $C_6H_9N_3O_2$ and its pH is around 6.5 (Fig. 3b). A histidine molecule is an amino acid with a ring-shaped imidazole group. This amino acid presents a strong interaction with metals because the electrons on the imidazole voluntarily form coordinate bonds with the metal. Therefore, his-tagged proteins can be immobilized and bind to different types of ions such as nickel and cobalt as seen in Fig. 3c [19].

D. Nickel Chloride

Nickel chloride ($NiCl_2$) is a water soluble compound with molecular weight of 129.959 g/mol. Structurally, the nickel atom bonds to three chlorines, embracing a similar structure to that of CdI_2 .

With certain acidic properties and a pH around 4 due to the hydrolysis of the Nickel ion Ni^{2+} , this compound has an ionic character which makes it a strong electrolyte capable of conducting electric current.

Regularly it is found as a yellow powder that when mixed in water changes to a green color. Because of nickel, this compound exhibits high affinity to his-tagged proteins, increasing non-specific binding with the surface of the protein.

III. MATERIALS

The main variables modified in the experiment were the concentration of nickel chloride and the purification of the sample. However, in this paper we include all the variations used to find the most beneficial outcome for protein attachment.

A. Biomolecules

Proteins or biomolecules are the biomarkers used in nano-electric devices to detect the presence of immunological responses due to anomalies. This experiment used three different his-tagged proteins to test the density of each of these in molybdenum disulfide. The three proteins used were:

1. Osteopontin (OPN)

OPN is a protein in charge of several tasks that include immunological response and tumorigenesis. It plays a big role as a prostate cancer biomarker that binds to the monoclonal antibody 23C3. In this study the concentration of the protein was of 20 μ M.

2. Anti - Herstatin 2 (Anti – Her 2)

In the experiment, Anti – Her 2 was used in a concentration level of 50 μ g/ml. This monoclonal antibody binds to Her2, a

type of protein that is found in on breast cells and that in abundance creates continuous growth, forming a tumor.

3. Insulin-like growth factor receptor 1 (IGFR-1)

This biomolecule is a receptor protein that controls the effect of IGF-1. A protein found in several types of cancer because it promotes metastasis. The concentration of protein used in this investigation was of 100 μ g/ml.

B. Nickel Chloride

The nickel chloride was obtained from Sigma-Aldrich in a powder form at 98 percent of concentration. The solution was prepared in distilled water to a concentration of 100 mM. The solution was then filtered using a 22 μ m filter assuring a more purified solution. Following this, the main mixture was diluted to concentrations of 50, 25 and 11mM.

C. Purification of the substrate

The substrate was cleaned before and after the protein attachment chemistry was applied in order to guarantee that the nickel chloride and the proteins would only interact with each other.

The first process of purification was annealing in a controlled argon/hydrogen environment. The purpose of using this method was to remove residue from the adhesive in the tape used to manually exfoliate the MoS_2 sheets.

Two annealing processes were used. Procedure one required a temperature of 200 C and concentrations of 100 sccm (standard cubic centimeter per minute) of hydrogen and 1000 sccm of argon for 2 hours. Procedure 2 used a temperature of 300 C in continuous emission of hydrogen and argon gas in a concentration of 20 sccm and 600 sccm respectively [12].

In between and after the protein attachment procedure, the molybdenum disulfide flakes were cleaned with water baths using a stirrer plate.

III. METHODS

The presence of a single layer of molybdenum disulfide exposed to the environment increases sensitivity. The ideal geometry of the molecule is approached when the fewest number of layers of the material are used. Therefore, the few-layer flakes were obtained by mechanical exfoliation from a crystal of MoS_2 by adhesive tape. These flakes were then immediately deposited on a silicon wafer of 500 μ m of thickness coated with a silicon oxide surface with gold alignment markers previously processed. Fig. 4a shows the silicon chips onto which the flakes of MoS_2 were exfoliated.

An inspection in the optical microscope was performed to find the thinnest flakes. Through optical images of the flakes we were able to distinguish a range of color from white to navy blue. The portion of the flakes in white color represented molybdenum disulfide in bulk form. However, as this color became darker the number of layers decrease. Our goal for instance, was to find the darkest flakes portrayed with a dark blue color. Fig. 4b displays a typical MoS₂ flake used in this experiment. These specific flakes were then scanned using atomic force microscopy (AFM) to ensure that the flakes were sufficiently thin and that the surface was clean and free of contamination.

Following this, the flakes with less contamination and fewest numbers of layers were used in the next steps. Nickel chloride was added to the chip with MoS₂ flakes for 30 minutes, making sure the solution covered the whole chip.

Four baths of five minutes each with distilled (DI) water were performed, assuring to eliminate NiCl₂ residue on the chip before the protein exposure. A final water bath in a spinner plate was then executed for seven minutes in a speed of 300 rpm.

The protein was added afterwards, making sure that the biomolecule would cover the surface for an hour. The MoS₂ flakes with proteins went through four DI water baths for five minutes each. The final bath was again performed in a spinner plate under the same conditions specified above.

Finally, the flakes were scanned using atomic force microscopy (AFM) in order to prove that the proteins were attached to the substrate.

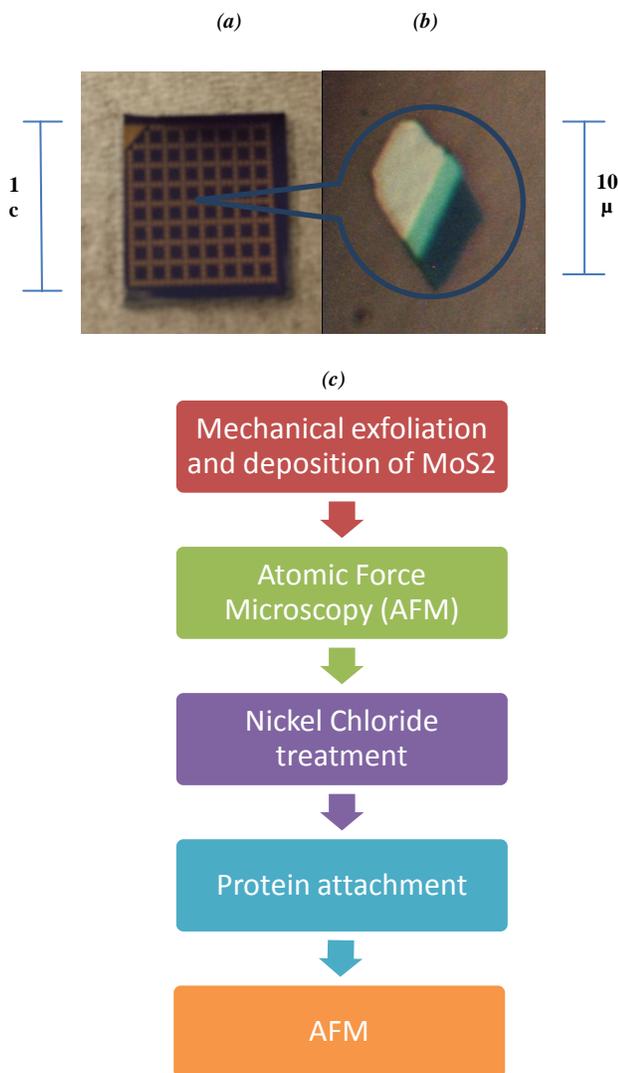


Fig. 4. (a) Silicon chip where exfoliated MoS₂ was deposited. Each chip was approximately 1cm per side. (b) Microscope image of an MoS₂ flake. The navy blue portion of the flake represents the fewest number of layers. The thickness of the flake and the layers of MoS₂ increase as the darkness of the color decreases. (c) Procedure timeline applied in the experiment.

IV. EXPERIMENTAL RESULTS

In order to analyze and assess the optimum nickel chloride conditions to attach proteins to molybdenum disulfide, the atomic force microscope (AFM) Dimension 3000 was used. The atomic force microscope is a machine able to scan a surface by tapping it with a tip on a cantilever that oscillates up and down, creating frequencies that differentiate the roughness of a surface. The interactions of the forces acting on the cantilever when the tip comes closer to the surface makes the amplitude of the cantilever decrease and control the force and height of the tapping. The image is then produced by the intermittent contact of the tip with the surface of the sample.

Results show interesting qualitative characteristics depending on the concentration used. Fig. 5a shows the AFM scan of an MoS₂ flake. The image displays two main regions with few layers of molybdenum disulfide pointed by the arrows. Fig. 5b shows the height profile in nm of the two thinnest regions. Region 1 is approximately 2.7 nm tall and region 2 is 4.5 nm. This corresponds to 4 and 5 layers of MoS₂ respectively.

In Fig. 5c we can see an AFM picture of the same flake zoomed in 66 percent. In this case the proteins (IGFR-1) have been attached to the flake using a nickel chloride concentration of 11 mM. Calculations present a protein density of 70 proteins per μm². In addition, a histogram is shown in Fig. 5d. It displays the height of the proteins attached. The highest frequency of proteins was found to be of height 4.1 nm.

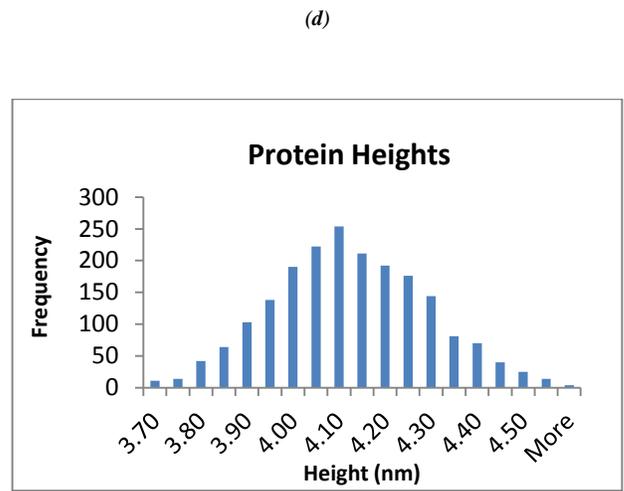
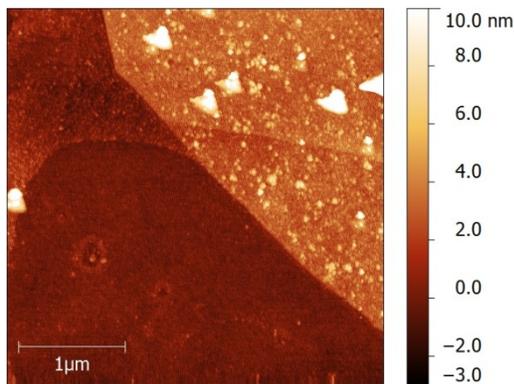
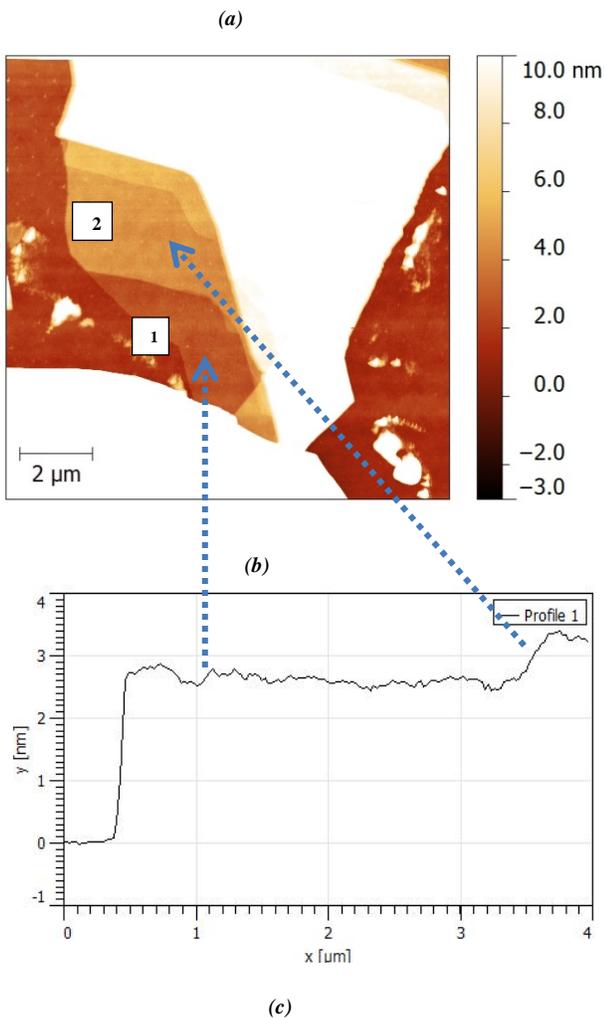


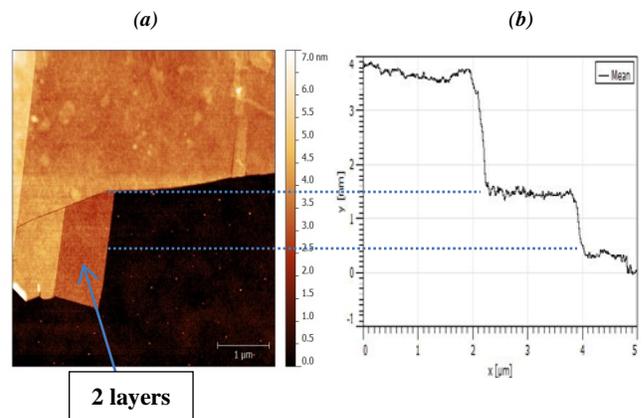
Fig. 5 (a) AFM scan of an MoS₂ flake displaying two major dark areas constituting the fewest number of layers. Region 1 displays 4 layers and Region 2 illustrates 5 layers. (b) Height profile of Region 1 and 2. (c) AFM scan of the same flake with proteins zoomed in 66 percent. (d) Histogram of the height of the proteins.

Furthermore, several observations were noticed at different concentrations. The results are shown below:

Nickel Chloride at 100 mM (Fig. 6)

At this concentration a large amount of residue from nickel chloride was found. Particles of size around 4-6 nm were spread all over the surface of the flake. However, none of these particles were seen in the rest of the silicon surface.

In addition, the overall thickness of the flake increased by 2 nm, which is a result of chlorine forming a layer of salts. Because of the similarity in height of the proteins to the height particles found, the experiment did not proceed to further steps.



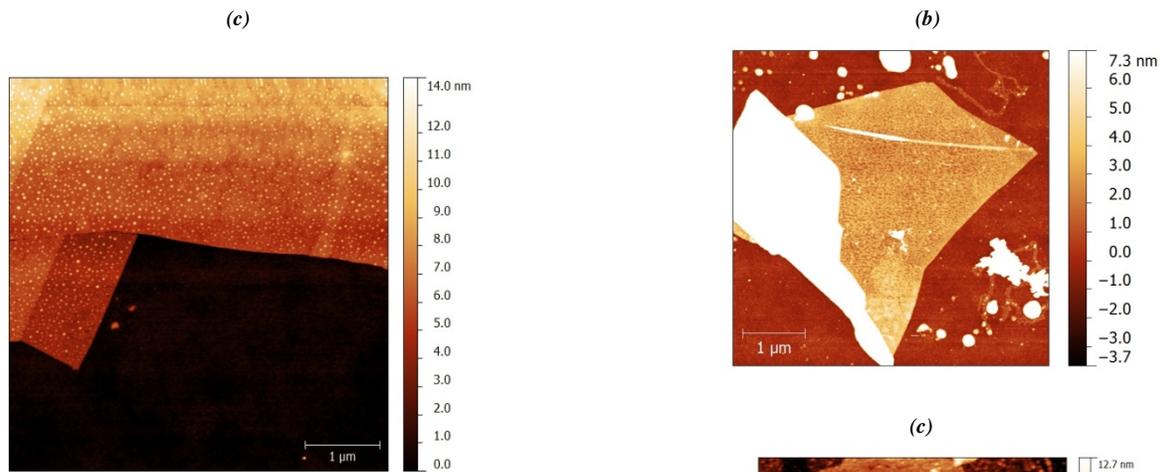


Fig. 6. (a) AFM picture of the thickness of the flake. The thinnest section of the flake, indicated by the arrow is about 1.5 nm thick or about 2 layers of molybdenum disulfide. (b) Profile of the average heights of the flake, starting with the thickest portion of the flake to the left area moving right and reaching very thin areas. (c) AFM scan of the same flake after nickel chloride treatment at 100 mM.

Nickel Chloride treatment at 15 mM (Fig. 7)

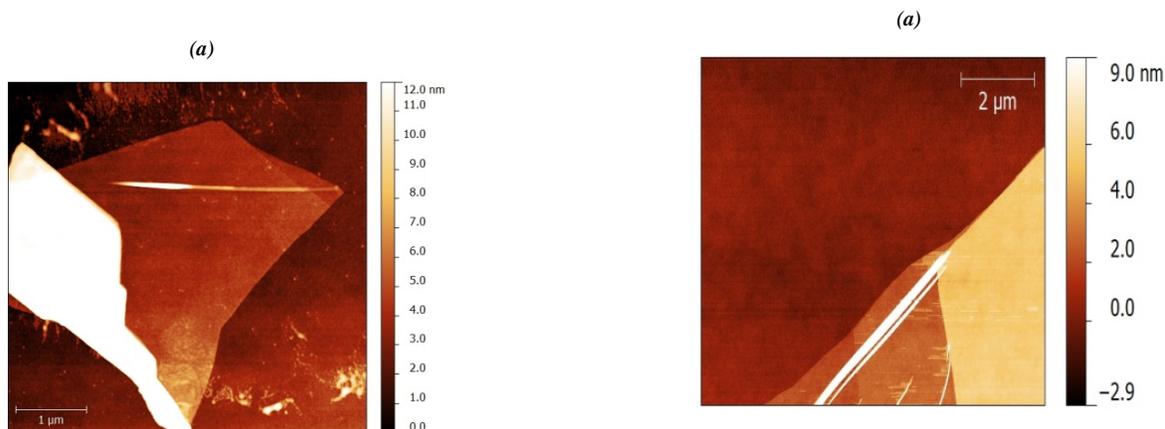
The flake used at this condition presents some residue on the silicon oxide surface. However, the flake looked clean as seen in Fig. 7a. After the nickel chloride treatment, there was a layer with holes formed on the substrate.

Furthermore, this layer was also thick enough to increase in 2 nm the overall thickness of the flake as portrayed in Fig. 7b. Additionally, the height of the holes was measured and these show that closer to the edges of the flake the adhesion of this layer is less. As the nickel solution is spread towards the edges, the thickness of these holes decrease and for instance the height of the layer of salts is smaller. On Fig. 7c the AFM scan with proteins is shown. As portrayed, there are fewer proteins in the molybdenum disulfide flake due to the layer of holes.

Fig. 7. (a) AFM picture of an MoS₂ flake. (b) AFM picture of the same MoS₂ flake that underwent nickel chloride treatment at a concentration of 15mM. (c) AFM picture after protein attachment.

Surface coated with aluminum oxide (Al₂O₃)

The addition of aluminum oxide to the silicon oxide surface of the wafer was used to see the effects of protein density distribution throughout a hydrophobic surface of Al₂O₃ underneath the molybdenum disulfide flakes. Fewer residues were found in these flakes. Results also show an interesting accumulation of proteins in the flake. Proteins in the MoS₂ show heights around 7-8 nm.



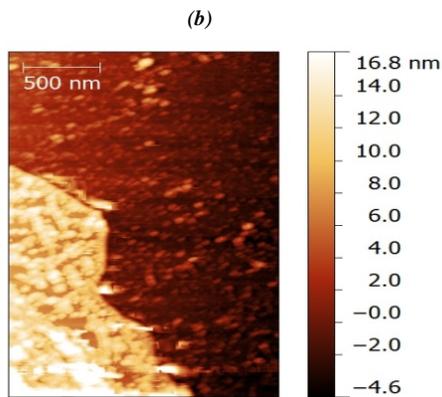


Fig. 8. (a) AFM scan of a flake of molybdenum disulfide in an aluminum oxide substrate. (b) AFM scan of the same flake with proteins attached. There is a high density of 3 proteins accumulated per cluster.

V. DISCUSSION AND CONCLUSION

We have successfully attached proteins to few-layer molybdenum disulfide flakes as the first step towards engineering a device for biosensing applications. Through this investigation we have found that a low concentration of nickel chloride used as a cross-linker is better for the chemical interaction between the molybdenum disulfide and the proteins. At a higher concentration of nickel chloride (>11mM) the imidazole group of each histidine gets to a point in which it can no longer create coordinate bonds with the nickel ion. In this case, there is no chemical interaction, and the nickel chloride excess remains laid on the surface either as a particle solution or as a porous layer.

For instance, the lowest concentration used in this experiment (11 mM) presented the highest density of proteins attached to the flakes compared to the substrate. Approximately the ratio of proteins between the MoS₂ and the silicon oxide/ aluminum oxide substrate was 5:1. When altering the concentration of NiCl₂, we saw major changes to the flake thickness and to its qualitative characteristics such as texture and roughness, affecting protein binding strength.

An important factor to consider is the residue formed by the adhesive tape. In this experiment, the use of flakes contaminated with this residue was avoided due to control variables in order to prevent the interaction of these substances with nickel chloride. However, if we were able to synthesize monolayers of molybdenum disulfide in a scalable fashion, for example by chemical vapor deposition, we would avoid contamination due to sticky residues which are hard to remove.

When using the alumina coated surface, a clustering of proteins was formed. This demonstrates the effects of hydrophobic surfaces in protein interactions. This surface forced proteins to cluster in small groups, showing an ununiformed density of proteins throughout the flake.

This research is promising and the next steps in the creation of a biosensor are to attach electrodes to the molybdenum disulfide flakes through electron-beam lithography and obtain the voltage-current relationship of the flake with proteins. Once we obtain this profile, we must attach the second protein to the receptor already found on the MoS₂ and proceed to characterize the electronic transport data. Following this, we must investigate the lowest concentrations of target protein that we are able to detect.

In the long run, research must be done regarding reproducibility and antigen to antibody binding. Furthermore, we should keep looking for more chemical attachment methods in order to improve the protein density ratio between the substrate and the flake. One possible option is to use an ion similar to nickel such as cobalt, a metal that increases specific binding.

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