

A NOVEL BIOSENSOR BASED ON A PLANAR WATER GATED
ORGANIC FIELD EFFECT TRANSISTOR USING BIOTIN
FUNCTIONALIZED POLYTHIOPHENE

by

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ABSTRACT

A NOVEL BIOSENSOR BASED ON A PLANAR WATER GATED ORGANIC FIELD EFFECT TRANSISTOR USING BIOTIN FUNCTIONALIZED POLYTHIOPHENE

In this thesis, a novel affinity type biological sensor based on a planar water-gated field effect transistor (WGOFET) is introduced and investigated. The liquid electrolyte layer in the WGOFET is used as the gate insulator and presented the opportunity to add water soluble biomolecules such as streptavidin to the transistor. It has a simple and low-cost fabrication process and can be integrated to microfluidic channels. It works below 1 volt. Devices are fabricated on a glass wafer by patterning the same Cr/Au (5nm/50nm) thin film to form source, drain and gate electrodes. Functionalized polythiophene polymers with biotin were synthesized and dissolved in chlorobenzene with a ratio of 5 mg/mL⁻¹. The polymer solution is then spin coated on the wafer to form the active layer of the device. Streptavidin solutions with three different concentrations of 0.4, 1 and 2.5 µg/mL are prepared and put as a droplet into the O-ring placed over the active region of the device. A cover glass is used to confine the droplet. I_{DS} - V_{DS} and I_{DS} - V_{GS} curves of the devices are measured at specific intervals for 30 minutes. A typical on-off current ratio of 220, threshold voltage of -0.2 V and mobility of $3 \times 10^{-3} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ are measured. On-current of 270 nA is achieved at -0.5 V V_{DS} and -1 V V_{GS} . The on-current value drops to 80 nA after 30 minutes of specific interaction between streptavidin and biotin molecules. The on-current values drop with the increased concentrations of streptavidin.

ÖZET

BİYOTİN İLE ETKİNLEŞTİRİLMİŞ POLİTİYOFEN KULLANAN YATAY SIVI KAPILI ORGANİK ALAN ETKİLİ TRANSİSTÖR TEMELLİ ÖZGÜN BİYOSENSÖR

Bu tezde, yatay sıvı kapılı alan etkili transistör (WGOFET) temelli yeni bir bağlanma tipi biyolojik sensör tanıtılmış ve incelenmiştir. WGOFET içindeki sıvı elektrolit tabaka kapı yalıtkanı olarak kullanılmaktadır ve transistöre streptavidin gibi suda çözünen biyomoleküllerin eklenmesi olanağını sunmaktadır. Bu sensörün basit ve düşük maliyetli bir üretim süreci vardır ve mikro akışkan kanallarla bütünleştirilebilmektedir. 1 voltun altında çalışmaktadır. Cihazlar bir cam levhanın üzerindeki aynı Cr/Au (5nm/50nm) ince filminin şekillendirilerek kaynak, savak ve kapı elektrotlarını oluşturması sonucu imal edilmiştir. Biotin ile etkinleştirilmiş politiyofen polimerler sentezlenip, 5 mg/ mL⁻¹ oranında klorobenzen içerisinde çözündürülmüştür. Polimer çözeltisi daha sonra cihazın aktif tabakasını oluşturmak için döndürmeli kaplamayla pulun üzerine kaplanmıştır. 0.4, 1 ve 2.5 µg/mL olarak üç farklı konsantrasyonda streptavidin çözeltisi hazırlanıp cihazın aktif bölgesi üzerine yerleştirilen O-halkası içine bir damla halinde konulmuştur. Bir cam kapak damlacığı sınırlamak için kullanılmıştır. Cihazların I_{DS} - V_{DS} ve I_{DS} - V_{GS} eğrileri 30 dakika boyunca belirli aralıklarla ölçülmüştür. Açık-kapalı akım oranı 220, eşik gerilimi -0.2 volt ve mobilite de $3 \times 10^{-3} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ olarak ölçülmüştür. -0.5 volt V_{DS} ve -1 volt V_{GS} gerilimleri altında açık-akım 270 nA olarak elde edilmiştir. Açık-akım değeri streptavidin ve biyotin molekülleri arasındaki özel etkileşim sebebiyle, 30 dakika sonra 80 nA değerine düşmüştür. Streptavidin konsantrasyonu arttıkça açık-akım değerleri düşmektedir.

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LIST OF SYMBOLS

Ab	Antibody
C	Gate insulator capacitance per unit area
D	Diffusion constant
I_{DSAT}	Saturation current of transistor
I_{DS}	Drain-Source Current
Ig	Immunoglobulin
I_{Off}	Off current of transistor
I_{On}	On current of transistor
kD_a	Atomic mass unit
K_D	Dissociation constant
q	Diffusion dimensionality constant
t_D	Diffusion time
V_{DS}	Potential difference between Drain and Source
V_{GS}	Potential difference between Gate and Source
V_{th}	Threshold voltage
x_D	Diffusion distance
ϵ_0	Permittivity of vaccum
ϵ_r	Relative permittivity
μ_{linear}	Mobility in Linear Region
$\mu_{saturation}$	Mobility in Saturation Region

LIST OF ACRONYMS/ABBREVIATIONS

BGBC	Bottom Gate Bottom Contact
BGTC	Bottom Gate Top Contact
CV	Cyclic Voltammetry
EDL	Electrical Double Layer
EDLC	Electrical Double Layer Capacitance
FET	Field-Effect Transistor
FFD	Fluidic Force Discremination
HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
MESFET	Metal-Semiconductor FET
MISFET	Metal-Insulator FET
OTFT	Organic Thin Film Transistor
TFT	Thin Film Transistor
TGBC	Top Gate Bottom Contact
TGTC	Top Gate Top Contact
P3HT	Poly (3-Hexyl) Thiophene
VRH	Variable Ranging Hopping

1. INTRODUCTION

1.1. Organic Electronics

The year, 1947, was a milestone in the history of electronics and technology. Scientists and researchers in the Bell laboratory invented transistor which was made of inorganic solid semiconductors. The device showed notable electrical characteristics and features showing great potential for amplifying signals and other electronics applications. After the invention, transistor as a hidden vital part for every electronic device started to dominate the whole electronic world. As today, we can observe and understand the existence of millions of transistors around us.

The main semiconductor material of the transistors nowadays is silicon. Although it has a lot of advantages like abundance, single-crystalline silicon requires high purification increasing its cost. This makes the silicon an inappropriate choice for large area and low cost electronic applications. As a solution, organic semiconductors were introduced and investigated.

A fundamental difference between inorganic and organic device is their fabrication processes. A conventional silicon chip takes weeks to produce and requires lots of effort and a clean, sophisticated laboratory. However, a device based on organic materials like plastic or a polymer film, which are non-crystalline and flexible materials, can be fabricated in a very short time in a simpler laboratory with cheap and low-temperature processes. The organic material's light weight and mechanical flexibility allow the researchers to use the general printing methods to produce large number of devices in a limited area in a very short period of time. Additionally, use of polymers and other organic materials make the chemical modification of the devices possible. The light emitting diodes, photo-active cells, and organic field-effect transistors are examples of devices which have taken the advantages of organic semiconductors to optimize their performance for specific applications. [1].

Due to some disadvantages of organic materials like poor crystalline structure, low mobility and possible degradation under environmental influence, they are unlikely to take

the place of inorganic materials in the modern electronic devices. However, they can be used in special and particular sensing applications. In this thesis, we mainly focus on the field of bio-sensing and study a novel water gated planar organic field-effect transistor as a biosensing device to detect the interactions between specific biomolecules.

1.2. Chemical sensing and Bio-sensing

Sensing as a branch of science and technology, is a field that employs sensing devices which are used to detect or measure a physical, chemical or biological property and record, indicate or otherwise respond to it. There are mainly three types of sensors: physical sensors, chemical sensors and biological sensors. Various definitions and terminologies are used for the biosensors depending on the field of application.

A biosensor in a more common and simple definition is an analytical device which is used mostly for recognition of target biological molecules, macromolecules, and atoms which can also be referred as analytes. Typically, a biosensor consists of three main components: a biological recognition part that is responsible for the specific interaction with analyte second one; a transducer which transduces the biological interaction to an electrical signal and an output system.

Organic field-effect transistors have shown significant potential for the field of chemical sensing and bio-sensing. The OFETs based on polymers are able to be integrated into sensing devices to meet the required conditions for more sophisticated sensing devices [2]. The field-effect based transistors have a good number of advantages which make them a great substitute for the traditional solid-state devices in the biosensors fields.

Employing organic materials like polymers as their active layer, ease of fabrication using low-cost low-temperature processes, ability to improve their physical and chemical sensitivity and selectivity by changing their molecular structures, possibility of introducing recognition elements into their active regions or bulk material plus ability to incorporate new materials to their electrolyte layers are of OFETs great advantages

making them a suitable choice for the matter of chemical or bio-sensing [3-7] OFETs have shown stable and reliable response in contact with aqueous media which allows the biological analytes to be introduced in a liquid-state [8]. Furthermore, signal amplification nature of a transistor allows an organic field-effect transistor to perform as a better sensing device.

A number of works have been done recently in the field of chemical and bio-sensing. OFETs have been utilized to measure humidity of the atmosphere around them [9, 10], airborne explosive vapors like DNT and TNT [11, 12], and to sense alcohol [13]. As a result of OFETs being compatible with liquid-state materials, new aqueous organic materials can be incorporated into the field-effect transistors. As another example, a recent study has investigated the detection of antibodies using a pentacene based field-effect transistor in a liquid-state media [14].

In this work, we pursue a novel planar organic field-effect transistor as a biosensor to detect and record analyte interactions. A synthesized polymer functionalized with receptors is used as the active layer for the OFET and ligands are added to the electrolyte layer. The electrolyte layer is in direct contact with active layer which provides a proper environment for the ligands to diffuse toward receptors and interact with them. As a model for ligand-receptor binding we have choose biotin-streptavidin proteins as were reported in a number of studies [15-17].

1.3. Outline and Objective

Organic Electronics as a new emerging field are explored in the sensing applications and have found a wide range of applications in different fields. Nowadays, OFETs are of great interest in the field of chemical and biological sensing. Their prominent advantages over the conventional transistors, such as solubility, simple and easy fabrication, low power consumption, high sensitivity and *etc.* have made them a prosperous and potential device for the role of sensors or transducers.

A crucial feature of the OFETs in order to be able to be used in biosensors is low voltage operation. Most of the previously reported OFETs have high driving voltages, which are considered to be damaging for the biomolecules. As a solution to this problem, the gate dielectric of organic field-effect transistors is replaced by an ion-conducting electrolyte, like DI water, on top of the semiconductor layer [18]. This layer provides a high dielectric constant resulting in a large double-layer capacitance (EDLC), so that the device can operate in relatively low voltages. Additionally, a liquid state dielectric layer can provide a bio-friendly environment for the biomolecules.

According to the mentioned outstanding properties of electrolyte-gated OFETs, we decided to build a simple and low-cost biosensor based on them. Biotin and streptavidin, have attracted attention of many research groups because of their high affinity and strong interactions. Therefore, these proteins were utilized to model a ligand-receptor interaction in this project. To form the semiconductor layer of OFET, polythiophene polymer was synthesized and biotin molecules were added to the polymer chains. Streptavidin molecules were added to the electrolyte layer and a droplet of solution was put over the active region of OFET.

After fabricating the OFET and coating it with modified-P3HT, a droplet of prepared solution was put over the active region and by taking contacts from transistor electrodes to a semiconductor analyzer, the electrical responses of the relative OFET were recorded.

The main goal for this project was to investigate a novel, simple and low-cost to fabricate, reliable biosensor to detect vital biological or chemical analytes.

In Chapter 2, a brief definition about the conjugated and synthesized polythiophene (P3HT) as the semiconductors will be given and afterwards details about structure and operation of organic-field effect transistors will be explained. In the following, we will be discussing about biological-chemical sensing and its various methods to give more details about this issue.

In Chapter 3, fabrication process of the water-gated OFET will be explained which will be followed by the explanation of biotinylated P3HT synthesis method.

In Chapter 4, the results of OFET behavior will be probed and analyzed. The transfer curves for each experiment will be given and the behavior of transistor under certain conditions will be investigated. In the following, the theory for the sensor operation will be discussed and statements will be given to validate the sensor behavior. And at the end the general behavior of the sensor will be explained.

In Chapter 5, a conclusion from the analyzed data and measurements will be drawn and feedbacks for the future works in this field for the bio-sensing applications will be given. Referring to the challenges and issues in the present work and previous works, we will investigate a new potential method for more sophisticated future work in the same topic of this thesis.

2. THEORY

2.1. Organic semiconductors

Organic electronics or polymer electronics are a branch of material science. The organic electronic devices have attracted many attention due to their notable properties: Low-cost, low-temperature fabrication processes, mechanical flexibility, ability to be printed over substrates through conventional printing methods. Devices like organic light-emitting diodes, photoactive cells, photo detectors, printed electronics, memory cells and organic field-effect transistors are examples of the relatively new developed organic devices.

One of the most important part of organic electronic device is the semiconductor polymer used in the active region. More details about the structure and the building units of the organic materials will be given in the next sections.

One of the main and in-developing organic electronics applications is the organic field-effect transistor. There are various types of the OFETs depending on the structure and semiconductor material that is used inside them. A unique property of organic electronics is their ability to be active and interact with aqueous media. This has made the organic field-effect transistor to be a potential candidate for the chemical or biological sensing applications. The polymer used in the OFETs can be modified and synthesized in order to attach desired biomolecules on its chains. Interactions between added molecules to polymer and the molecules in surrounding environment are expected to impact the electrical features of the OFET. As a result, organic field-effect transistors have the potential to operate as a biological or chemical sensor.

2.2. Organic field-effect transistors

Over the past 20 years, organic FETs have dominated a great area of research. To simply describe an OFET, we can say it is a transistor in which an organic semiconductor is used as its channel. Although the organic semiconductor's electronic mobility is relatively lower than that of inorganic semiconductors, their unique properties like low-budget fabrication, light-weight and mechanical flexibility have intensified their role in the modern electronics. The p-type organic devices are of more interest in the new applications because of their high hole mobility which is reported to be close to the hole mobility of amorphous silicon [19, 20].

The very first articles about the organic FETs as a potential device for electronic applications were reported in the 70's [21-23]. However, a research in 1987 by Kezu demonstrated a practical use of an electrochemically synthesized polymer in a field-effect transistor and confirmed their normal field-effect behavior [24]. The performance of organic FETs have been improved continuously throughout the years and several reviews have been reported recently [25-27]. The OFETs are designed in various geometries and structures and various materials can be employed as their semiconductor channel or insulator layer.

Due to the organic semiconductors prominent advantages over the inorganic ones, they have found numerous applications in industrial, chemical and also medical and biological sensing fields. Glycogen sensor is an example of the biological sensors based on organic field-effect transistors [28]. OFET based devices promise good candidates for disposable sensors in medical or biological applications.

2.2.1. Principle of operation

In general, transistors which are essential and basic components of every circuit and electronic device are used as signal amplification component or on-off switches. The operation of an OFET as a transistor is based on the field-effect phenomenon. This

phenomenon is described with the change and control of conductivity in the transistor semiconductor layer (channel) by an applied electrical field normal to its surface [29].

OFETs like other field-effect transistors consist of three main terminals which are source, drain and gate as well as semiconductor layer and insulator layer. Often, source electrode is grounded and electrical field is applied to the gate. This field controls the shape and conductivity in the channel and creates a current flow between source and drain electrodes. Whenever the conductive channel of charge carriers is generated between source and drain, the transistor is considered to be on and current flows through the conductive channel. A reverse voltage applied to the gate, causes the conductive channel to close and hence prevent the current to flow.

In the p-type semiconductor based OFETs, which are more common because of the higher hole mobility, a negative voltage greater than the threshold voltage of the device is applied between gate and source terminals to cause accumulation of holes in the channel close to the gate electrode and create conductive p-channel in the semiconductor layer. For the current to flow in the channel a negative voltage is also applied to the drain terminal as well. In the result, a negative current starts to flow from drain to source and the magnitude of the current is controlled by the magnitude of the voltage applied to the drain of the transistor. The current can be increased by increasing the voltage until the drain current reaches its maximum amount and p-channel saturates. The maximum current is known as saturation current and its magnitude depends on the gate-source voltage.

2.2.2. Electrical characteristics of OFETs

The vertical electrical-field between gate electrode and semiconductor layer contributes to a capacitor. The two plates for the capacitor are the gate and semiconductor layer and the gate insulator layer is the dielectric of the capacitor. Thus, the capacitance per-unit of area can be calculated by the formula below in which d is the dielectric thickness, ϵ_0 is the permittivity of vacuum, and ϵ_r is the relative permittivity of semiconductor layer.

$$C = \frac{\epsilon_0 \epsilon_r}{d} \quad (2.1)$$

The essential condition for a field-effect transistor to operate is that the applied gate to source voltage, which is negative in p-type transistor and positive in n-type, exceeds a minimum voltage, which is required to form the conductive channel. This voltage is called the threshold voltage and is shown by V_{th} . The Equation 2.2 describes this condition for a p-type semiconductor based OFET.

$$V_{GS} < -V_{th} \quad (2.2)$$

After full-filling this condition, if V_{DS} voltage is applied to the drain and source electrodes, the I_{DS} current will flow from drain to source in the case of a p-type semiconductor. OFETs show flow of small off-currents in the result of their intrinsic charge carriers in semiconductor layer when there is no voltage applied to the gate electrode. One of differences of the organic field-effect transistors compared to ordinary field-effect transistor is the lack of an inversion region in order to isolate the active region from the bulk. Since, there is no need to invert charge carriers in the OFET, it operates in the accumulation region. So, the threshold voltage solely depends on the small shallow traps near the semiconductor interface and the work-function difference between gate and semiconductor [30, 18].

Organic FETs operate in two main regions: linear region and saturation region. The behavior is much like the behavior of a conventional MOSFET. The conditions and the formulas for the drain current in each operational region are given below. C in the following equations stands for gate insulator capacitance of transistor per unit area. W and L are the width and length of the transistor, respectively, and μ is the mobility of the semiconductor.

Linear region:

$$|V_{DS}| < |V_{GS} - V_{th}| \quad (2.3)$$

$$I_{DS(linear)} = \frac{W}{2L} \mu C \left[(V_{GS} - V_{th}) - \frac{V_{DS}^2}{2} \right] \quad (2.4)$$

Saturation region:

$$|V_{DS}| \geq |V_{GS} - V_{th}| \quad (2.5)$$

$$I_{DS(saturation)} = \frac{W}{2L} \mu C (V_{GS} - V_{th})^2 \quad (2.6)$$

Figure 2.1 depicts a normal and expected behavior of an OFET. The linear and saturation regions for different drain-source voltages are shown. The figure helps to better understand the behavior and operational regions of the OFET.

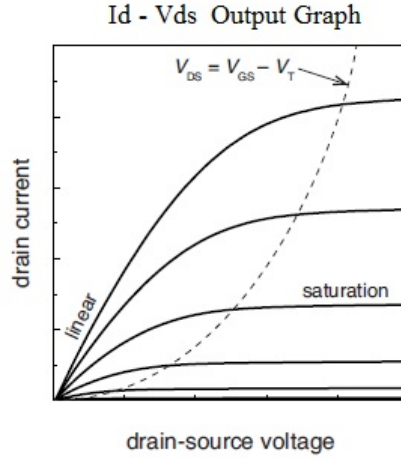


Figure 2.1. $I_{DS} - V_{DS}$ Transfer characteristic of an ordinary OFET [31].

Researchers in the field of OFET seek to optimize three main characteristics: On/off current ratio, threshold voltage and the charge carrier mobility. The on/off current ratio which is the ratio of drain current when V_{GS} is maximum to the drain current when V_{GS} is equal to zero. This value shows how the drain current can be modulated by the gate to source voltage. The equation for this ratio is given in the following:

$$\frac{on}{off} ratio = \frac{I_{on}(V_{GSmax})}{I_{off}(V_{GS} = 0)} \quad (2.7)$$

There are different methods for the calculation of threshold voltage. A common and simple way is to extract V_{th} from the $V_{GS} - I_{DS}$ transfer curve by plotting V_{GS} against square root of I_{DS} as it is shown in Figure 2.2.

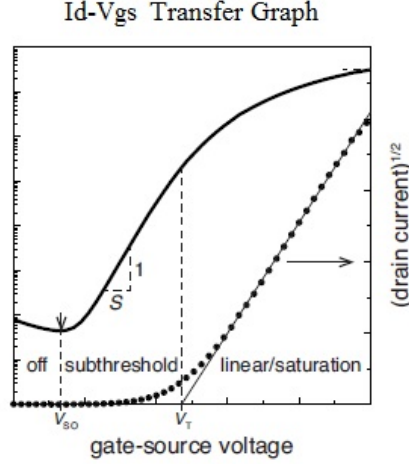


Figure 2.2. $I_{DS} - V_{GS}$ Transfer characteristic of an ordinary OFET [31].

Carrier mobility can be calculated for each of operational regions: linear and saturation. Transconductance value is essential for the calculation of the carrier's mobility. By using the transconductance definition and formula which is given in equation 2.8 and rearranging it, one can extract the mobility carrier value for both linear and saturation regions.

Transconductance:

$$g_m = \frac{\partial I_{DS}}{\partial V_{DS}} \quad (2.8)$$

In the linear region:

$$g_m = \frac{W}{L} \mu C V_{DS} \quad (2.9)$$

$$\rightarrow \mu_{linear} = \frac{L g_m}{W C V_{DS}} \quad (2.10)$$

And for the saturation region mobility:

$$g_m = \frac{W}{L} \mu C (V_{GS} - V_{th})^2 \quad (2.11)$$

$$\rightarrow \mu_{sat} = \frac{L g_m}{W C (V_{GS} - V_{th})^2} \quad (2.12)$$

So, the given formulas can be used to calculate the charge carriers mobility in both linear and saturation regions.

2.2.3. OFETs structures

Field-effect transistors are constructed in three major structures: MISFET, MESFET and TFT. A Metal-insulator FET (MISFET) is a transistor in which drain and source sections are connected by a semiconductor layer and the gate electrode is separated from the channel by an insulator layer. Figure 2.3 shows a schematic of a MISFET structure. One of the main advantages of the MISFET structure is that the depletion region between the p-type substrate and both the n-channel and the n⁺ regions below the source and drain contacts provides an isolation from any other device fabricated on the same substrate.

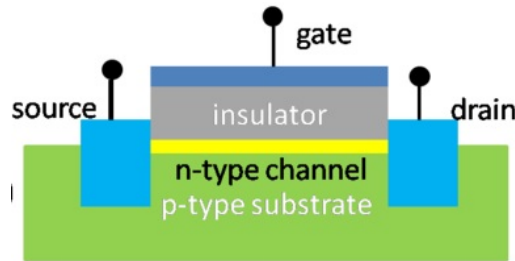


Figure 2.3. MISFET structure [32].

As another FET geometry, MESFET has only one difference from MISFET: the n-type source and drain contacts are connected by an n-type region. The channel thickness is partially reduced by the depletion region that forms at the Schottky gate electrode, the thickness of which is controlled by the voltage applied to the gate. In an off device, the

depletion region extends all over the n-type channel at zero gate voltage, whereas in a normally on MESFET, a portion of the channel is not depleted, thus ensuring the passage of a current at zero gate voltage [33]. Figure 2.4 depicts a simple MESFET structure.

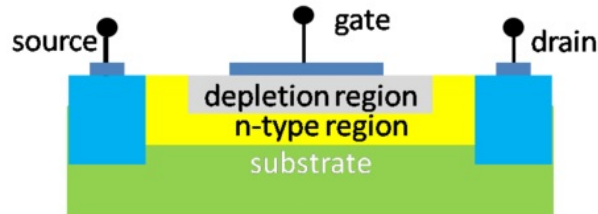


Figure 2.4. MESFET structure [32].

TFT or thin-film transistors were initially introduced in the 1986 by Paul Weimer [34]. In this particular structure, the source and drain contacts are directly deposited over the semiconductor layer and make ohmic contacts to the channel. Afterwards an insulator layer is deposited between the semiconductor layer and the gate electrode. This structure is well-suited for low-conductivity materials and is used in amorphous silicon transistors. Unlike the structures described above, here there is no depletion region to isolate the device from the substrate. As a unique property, TFTs operate in accumulation regime and not in the inversion regime. Figure 2.5 illustrates TFTs structure.

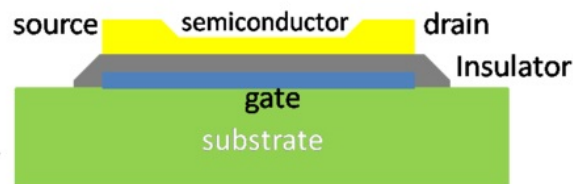


Figure 2.5. TFT structure [32].

The TFT structure is the most adopted structure for the organic field-effect transistors. Currently a common substrate on which to build organic TFTs is glass, since the primary application of OTFTs is in liquid crystal displays. With the increasing demand for flexible electronics, plastic substrates such as PEN are becoming more common. This differs from the conventional FET, where the semiconductor material such as silicon is the substrate.

In OTFTs, there are four different architectures determined by the locations of the gate as well as the source and drain electrodes within the material stack:

Top-Gate, Top-Contact (TGTC) TFT: Here the gate is farthest away from the substrate (i.e. the last layer to be applied), and the source and drain are placed at the top of the dielectric-semiconductor interface. Figure 2.6 shows the TGRC structure.

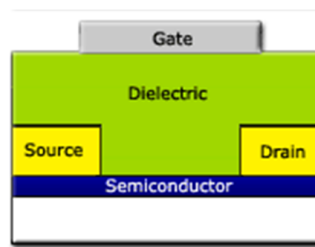


Figure 2.6. Top-Gate, Top-Contact [35].

Top-Gate, Bottom-Contact (TGBC) TFT: Here the gate is still the outer-most layer, but the source and drain are now adjacent to the semiconductor-substrate interface. Figure 2.7 illustrates this structure.

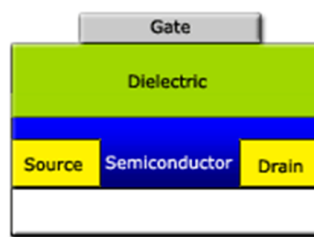


Figure 2.7. Top-Gate, Bottom-Contact [35].

Bottom-Gate, Bottom-Contact (BGBC) TFT: Here the gate is embedded within the dielectric atop the substrate, and the source and drain are at the semiconductor-dielectric interface. Figure 2.8 depicts A BGBC structure.

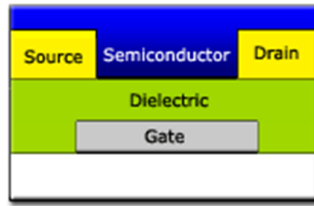


Figure 2.8. Bottom-Gate, Bottom-Contact [35].

Bottom-Gate, Top-Contact (BGTC) TFT: Here the gate is embedded within the dielectric atop the substrate, but the source and drain are now applied on top of the semiconductor layer. Figure 2.9 corresponds to a BGTC structure.

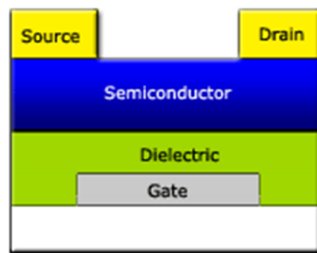


Figure 2.9. Bottom-Gate, Top-Contact [35].

With each architecture having its pros and cons, the choice of device architecture often has real consequences. For example, TGBC devices offer a large injection-face (i.e. the surface from which the charge-carriers leave the source and enter the semiconductor). However, processing-wise, BGBC may be preferred, since the devices with the semiconductor layer as the last one has historically been the easiest to produce. This is due to that the semiconductor layer does not have to be exposed to the potentially damaging chemicals needed to process subsequent layers.

2.2.4 Gate insulators in the OFETs

There are two major gate insulators that have been utilized in the OFETs structures: Conventional insulators and electrolyte based insulators. In early organic

electronic devices conventional inorganic gate insulator materials like SiO_2 ($\epsilon_r = 3.9$) were grown and combined with organic semiconductors, because the deposition process of inorganic gate insulators was convenient. A disadvantage of using SiO_2 as the gate insulator is the increased OFET operational voltage. A review has reported a working voltage of about $-40V$ for an OFET using SiO_2 as its insulator layer [36]. The solution to this problem is to reduce the gate-insulator capacitance to decrease the operational voltage of OFET. This can be achieved either by making the insulator layer thinner or using an insulator material with higher dielectric constant. In a recent work, a very thin layer ($3.5nm$) of SiO_2 have been incorporated, which has caused the OFET to operate in low voltages around 1.6 Volt [37]. High dielectric constant material like tantalum oxide and titanium oxide have been utilized to achieve low-voltage behavior in electronic devices.

Although the ordinary gate insulators are easy to incorporate into organic electronics, their low capacitance values per area cause the OFETs to switch on in large voltages. Recently, electrolytes as gate insulators have been introduced and utilized in organic FETs [38]. These insulators have much higher dielectric constant and create a better coupling of gate-electrode to the semiconductor layer. Electrolytes as gate insulators of OFETs form the electrical double layer capacitances (EDLC) which increases gate capacitance per area and reduce the driving voltage needed to turn the transistor on(details will be explained in the following section) [39]. Electrolyte-based dielectrics are considered to be inert and bio-friendly materials which can make the organic field-effect transistors as a potential biological or chemical sensors [40]. A simple and convenient electrolyte to be used as gate insulator layer is deionized water which is already available in every single laboratory. A recent work has investigated an organic field-effect transistor gated with a droplet of DI water which shows promising results [18]. Water gated OFETs or WGOFETs provide a stable and simple setup for the experiments in the field of biological and chemical sensing. In this project, because of its unique advantages, we have designed and fabricated a WGOFET as a sensing device.

2.2.5 Electrical Double layer

Whenever a metallic surface or electrode comes to direct contact with electrolyte, electrical double layer (EDL) forms in the interface of two separate phases. In the time that solid electrode is connected to the aqueous solution, charge poles will be distributed relative to each other over a short distance. This leads to the EDL phenomenon.

When a water solution is used as the gate insulator layer in an OFET, the electrical double layer is formed in the vicinity of the gate electrode. This thin layer contributes to a capacitance whose two plates are the gate electrode and the semiconductor layer, when there is a voltage applied to the gate terminal of the OFET. The electrical double-layer capacitance (EDLC) is relatively large capacitance which reduces the operational voltage of the OFETs significantly.

The details of the EDL phenomenon are illustrated in Figure 2.10. The basic principle to understand the EDL is to recognize that the boundary layer which is formed by ions or charged particle uniformly distributed and concentrated in the liquid near the charged surface is different from the extended solution. For better understanding, Gouy-Chapman double layer model will be explained. This model suggests that the applied voltage to the gate electrode attributes to the accumulation of ions with opposite charge at the interface. This layer is called first Helmholtz layer and the second one is formed at the interface of electrolyte and semiconductor layer due to gathering of repelled ions from metal-electrolyte interface. Diffusion of counter-ions from the bulk toward the Helmholtz layer creates a layer which is called diffusion layer and contains less amount of opposite-charged particles in compare to Helmholtz layer. As a summary, the Gouy-Chapman theory describes a rigid charged surface, with a thin layer of opposite-charged particles at the boundaries as well as a cloud of oppositely charged ions in the solution, the concentration of which decreases with distance from the surface.

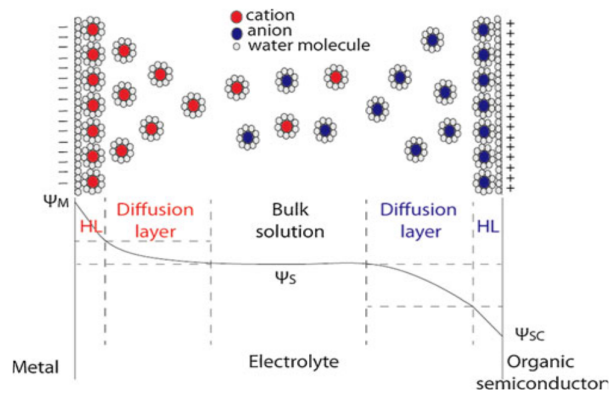


Figure 2.10. Illustration of EDL phenomenon and formation of Helmholtz layers (HL) in the interfaces and Diffusion layers within the electrolyte layer [3].

The EDL total capacitance that couples the metal electrode (gate) to the OS (organic semiconductor) is the sum of two series capacitances, which Helmholtz and diffusion layers contribute to their dielectric layers. Therefore, the whole EDLC will mostly depend on the value of the smaller capacitance which has thicker dielectric layer (diffusion layer). However, if the electrolyte layer concentration would increase, the diffusion layer thickness will reduce. This will cause the diffuse layer capacitance to increase and therefore, EDLC's value will get closer to the Helmholtz layer capacitance [41].

2.2.6 Planar Water-gated OFET

In this project, we have designed and fabricated a novel structure based on TFT geometry to create a simple and efficient OFET. As mentioned before, because of the great advantages of organic electrolytes such as high EDL capacitance and possibility of introducing biomolecules into it, we have used a droplet of DI water as the gate insulator for the OFET. Low-cost and low-temperature fabrication processes, ability to be produced in less than one day, unprecedented structure and highly reliable performance are of significant properties for the planar OFET. In this particular structure, gate electrode is not placed over the source and drain electrodes, but over the same substrate which is made of glass. Figure 2.11 depicts our special planar structure. As shown in the figure, the channel of the OFET is a narrow area between source and drain electrodes and the gate electrode

with two arms in both sides of active region induces the electrical field to the semiconductor layer generating the conductive channel between drain and source electrodes. The droplet shows the electrolyte layer that is in contact with gate electrode arms, source and drain electrodes. Further details about the water-gated OFET and its fabrication process and behavior will be explained in the next chapter.

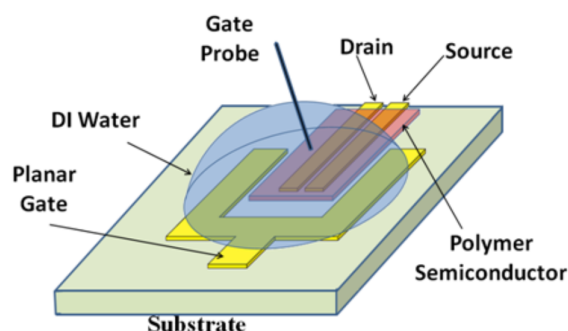


Figure 2.11. Planar water gated OFET [42].

2.3. Polymers as semiconductors

Polymers were known as insulator materials, until a study investigated over their conductivity and electrical applications [43]. Polymers attracted enormous interest as semiconductor and conductive materials. These new-emerging semiconductor materials show electrical properties close to those of inorganic semiconductors as well as high mechanical flexibility. One of main differences between polymer semiconductors and inorganic semiconductors is the charge generation and charge transport mechanism.

Organic semiconductors are broadly classified into two main classes: small molecules or oligomers and conjugated polymers. The basic and main building block for organic semiconductors is the carbon atom. Carbon can form four bonds with its neighboring carbon atoms or other atoms which is called s-bond or σ -bond. In the formation of simple molecules like CH_4 , each of the four electrons occupy four sp^3 hybridized orbitals and can form four covalent bonds with the neighbor carbon or other

atoms. However, in majority of complex molecules, three localized electrons occupy three sp^2 hybridized orbitals in a plane and the remaining electron occupies p_z orbital which is perpendicular to the sp^2 orbitals plane. In this case, sp^2 hybridization occurs in the carbon atom [44]. Whenever two or more sp^2 hybridized carbon atoms with one electron occupying p_z orbital are close to each other and an s-bond is formed between them by a formation of orbital overlap of two sp^2 bindings, the delocalized electrons in p_z orbitals form a weak bond which is called π -bond. Figure 2.12 illustrates the formation of so-called π -bond between two adjacent carbon molecules.

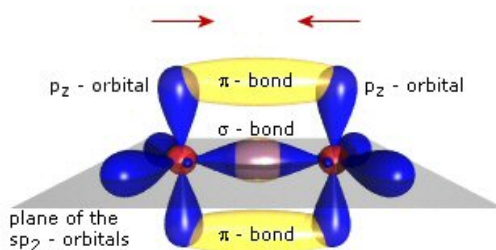


Figure 2.12. Two sp^2 hybridized carbon atoms and their orbitals and bonds [45].

The electrons in the π - bonds create a cloud of free electrons which are responsible for the conductivity and opto-electronic behavior of organic semiconductors based on carbon atoms. Semiconductor polymers are conjugated polymers which are formed by alternating single and double bonds of carbon atoms at their backbone. Figure 2.13 depicts how a conjugated polymer is formed and p_z orbitals overlap to create π - bonds.

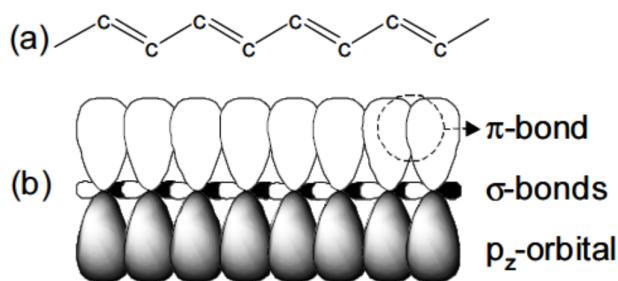


Figure 2.13. (a) A schematic of a conjugated polymer formed from single or double chemical bonds between carbon atoms (b) Delocalized electron densities that have formed π - bonds in the top and bottom of sp^2 orbitals plane [44].

In the organic semiconductors, molecular levels exist similar to the band structure in the inorganic semiconductors. Highest occupied molecular orbital or HOMO which refers to the valance band and the lowest unoccupied molecular orbital or LUMO refers to the conduction band in inorganic semiconductors. The gap between these two molecular levels contributes to the insulating properties of conjugated polymers [45].

Conjugated polymers have shown higher molecular weight and lower hole mobility in compare to the small semiconductor molecules due to their longer chains of connected molecules. Semiconductor polymers cannot be deposited by ordinary thermal evaporation methods, because they cannot tolerate the extensive increase in the temperature and the structure will be deformed. However, because of their good solubility in various solvents, they can be deposited by spin-coating or ink-jet printing methods which are much simpler and cheaper in compare to the other deposition processes.

2.3.1 Poly (3-hexylthiophene) as a conjugated polymer

Having unprecedented electrical and chemical properties, Poly (3-hexylthiophene) (P3HT) as a p-type thiophene-based conjugated polymer has been widely studied and investigated in the recent years. The chemical structure of the P3HT polymer as a member of polythiophene family which is used as the semiconductor material in this project, is given in Figure 2.14. It consists of thiophene backbones and hexyl side-chains (C_nH_{2n+1}) which causes the polymer to be soluble in organic solvents and can be attached to the 3 positions of thiophene rings. Major advantages of P3HT that have made it a good choice for low-cost plastic electronics are its good solubility and processibility, wide commercial availability, structural tunability and high charge carrier mobility reported up to $0.1\text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ [46-48]. Although, P3HT tends very much to interact with oxygen atoms in its surrounding environment [49, 50] which is not very desirable and affects the performance of devices, it is still considered as one of the stable polymers in the normal atmosphere.

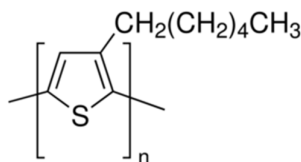


Figure 2.14. Chemical structure of P3HT [42].

The electrical properties of P3HT like conductivity and mobility are reported to be enhanced by means of structural modification [48], molecular weight [51] and process solvent type [52, 53]. The solvents play a key role in determining the polymer properties. A high boiling point solvent which is reported to enhance the crystal structure and mobility is chlorobenzene [45]. Consequently, we have selected P3HT as the semiconductor material for the WGOFET and prepared the polymer solution in chlorobenzene solvent.

2.3.2 Charge transport in conjugated polymers

Charge transport mechanism in the semiconductor polymers have been studied for over than two decades and there is still no specific and exact model describing the charge transport mechanism in the conjugated polymers. Nonetheless, there are some models reported to understand the whole process.

The issue with organic semiconductor polymers is that they do not possess long-range energy levels similar to those in organic semiconductor molecules. The reason for this is that the polymer chains are connected to each other by weak van-der-Waals forces and the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular weight (LUMO) have limited small ranges and can be easily disordered and separated in the result of non-conjugated carbon atoms or structural defects in the polymer chains [46]. So, the conjugated polymers do not have exactly two energy levels separated by a specific gap and the charge states are distributed over the polymer. The distribution of these charge transporting states are reported to be a Gaussian distribution [54].

The charge transport mechanism in the conjugated polymers are mainly described by two hypothesizes. In these theories the charge transport and semiconductor properties

depend on the morphology and irregularity in the structure and energy levels in polymers [45]. The first theory is the hopping transport mechanism, which is based on phonon-induced electron hopping from impurity state to another donor site with a vacant fraction. This basic theory was initially discussed for semiconductors with low impurity concentrations at low temperatures by Miller and Abrahams [55]. When this theory is applied to the semiconductor polymers, the disordered conjugated sites of polymer play the role of adjacent impurity site between which charge carriers would jump. Figure 2.15 shows how an electron hops from donor sites between two polymer chains. Furthermore, as Figure 2.15 illustrates, the vibration of polymers due to the increased temperature can reduce the gap between the impurity states with localized electrons which will enhance the mobility in the polymer.

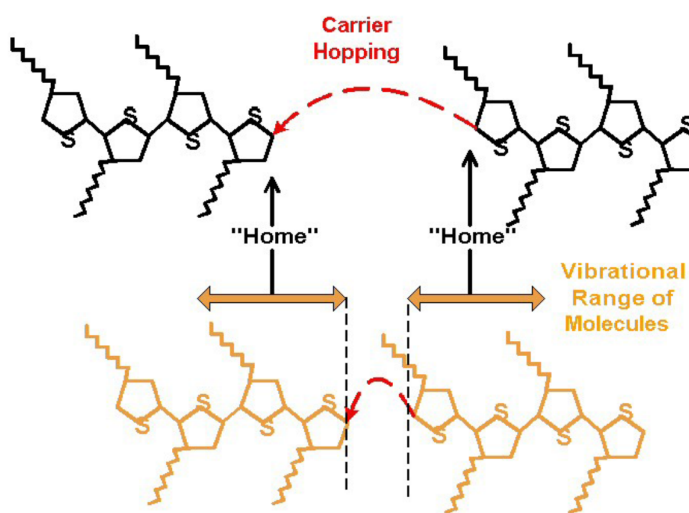


Figure 2.15. Charge transport mechanism illustration: when two polymer molecules have no vibration, the carriers need to jump a longer distance between sites. However, vibrational energy may bring two molecules close to each other resulting in less gap between sites for the charge carriers to hop [55].

It can be concluded from this mechanism that increase in the temperature would increase the molecules vibration lowering the gap between the localized states. This would increase the conductivity and the mobility in the conjugated polymers. There are other theories based on carrier hopping mechanism, which explain the charge transport through variable range hopping (VRH) [56]. Additionally, Vissenberg and Matters have suggested

a model based on VRH to investigate the impact of thermal energy and applied gate voltage (in organic field-effect transistors) over the charge transport between localized states [57].

The other mechanism used to interpret charge transport in polycrystalline semiconductor layers is called multiple trapping and release mode. The model considers the semiconductor layer to be consisting of crystallites separated by grain boundaries. The charge carriers in this model are able to move in the localized states in the crystallites; however they may be trapped into the de-localized states in the grain boundaries. These trapped carriers can be activated and be released from de-localized states in the result of thermal energy. After the releasing of trapped carriers, the mobility will depend mostly on the applied gate voltage in the transistor and because of this property this mobility is called as the field-effect mobility [58]. The trapping and release mechanism is very similar to that of charge transport in hopping model and the only way to separate these two mobility mechanisms is to use the Hall effect experiment [59].

2.4. Biosensors

Putting together the recognition ability of biomolecules and the good sensitivity of electrochemical devices led to the development of biological sensors. Tremendous amount of effort has been put into the investigations and studies over this subject [60, 61]. A biosensor is an analytical device which is used for analysis of bio-material samples to achieve an understanding of their bio-composition, structure and function by converting a biological response to an electrical signal. Figure 2.16 shows major elements in a biosensor.

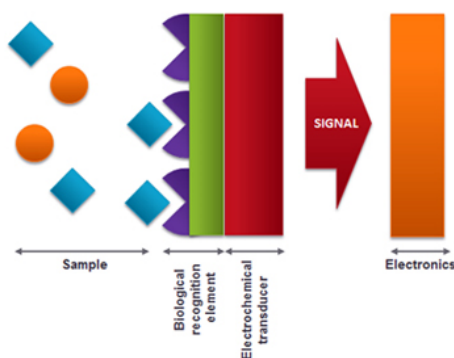


Figure 2.16. Diagram of the main sections in a simple biosensor device [62].

A biosensor consists of three parts: first, the analyte or substrate, which are sensitive biological elements immobilized over the surface of sensor by special immobilization techniques. These biological materials can be organ-cells, receptors, enzymes, antibodies and *etc.* The second part, is the transducer which detects the change in the biological layer and converts it to an electronic signal whose features depend on the concentration of specific biochemical or group of biochemical materials. And finally, an output system is composed of electronic circuitry and signal processing elements, which are responsible of showing the results in a user-friendly way. This part sometimes is known as the actuator.

Biosensors can be classified by their different recognition parts and transducers. The transducer parts of the biosensors are mostly classified by optic, acoustic, thermal, micro-cantilever based and electrochemical methods. The latter consists of potentiometric, voltammetry, conductimetry, and FET-based measurements. In these transducing methods changes in the biological layer is sensed by measuring voltage, current and conductivity of the electrodes or a transistor output characteristics

The very first biosensor was reported in 1962 by Leland C. Clark who is known as the father of bio-sensing [63]. He studied the electrochemistry of oxygen gas reaction at platinum metal electrodes. These electrodes were used as an oxygen sensor. The idea was to place an enzyme that would react with oxygen close to surface of the platinum electrodes by holding the enzyme in place with a piece of dialysis membrane. After this invention, because of their high potential, biosensors have found numerous applications. They are used these days in the clinical and non-clinical sensing fields. Pregnancy tests,

glucose monitoring devices [64], and infectious disease biosensor are of examples in biomedical sensing fields. In addition to their applications in health care, they are widely utilized in the control of industrial products and environmental monitoring as well.

2.4.1. Classification of biosensors based on receptors

Every biosensor can be divided into two major classes according to its bio-receptor section type. These two classes are known as the enzymatic (catalytic) and affinity based biosensors. In the first class, the biological elements are normally composed of enzymes which are immobilized with viable methods over the transducer surface. These biochemicals, which are in close proximity of analytes, create a chemical event which is converted to an electronic signal. The C. Clark sensor is categorized as an enzymatic sensor in which the entrapped glucose oxidase over the electrode would sense the concentration of the oxygen in its close environment. This concentration depends on the concentration of glucose molecules in the vicinity of the enzyme electrode.

Generally, the enzymes in catalytic sensors consume oxygen or produce the hydrogen peroxide during the catalytic event that takes place over the sensor substrate [65].

The other types of biosensors are the affinity-based biosensors. These sensors take advantage of interactions between biomolecules in order to detect and measure the target analytes. In other words, the immobilized biological part binds specifically with its counterpart and creates a complex (*e.g.* antigen binds to the antibody and forms a strong bond). This complex can impact energy levels in the substrate or change some of physical, electrical, optical or electrochemical characteristics of it. Figure 2.17 illustrates how the binding procedure happens in an affinity-based biosensor. At the first step the surface of sensor substrate is exposed to the sample containing both interested analyte and other particles. Secondly, followed by exposure of the analytes, they bind to the receptors and impact the substrate features. And at the end, a transducer detects the change in the characteristics of the substrate and translate it to an output electrical signal.

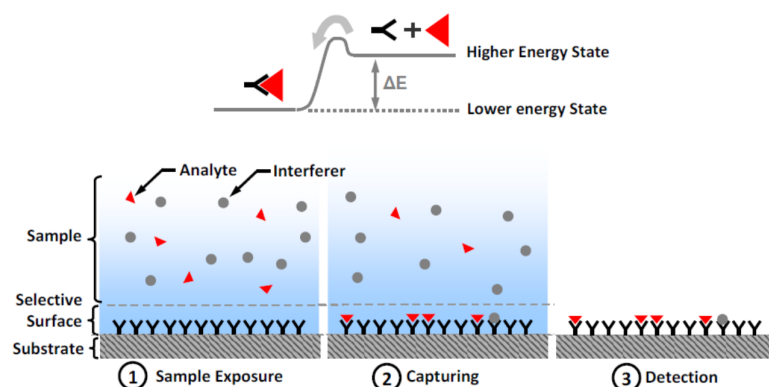


Figure 2.17. The basics of an Affinity-based biosensor surface [66].

One of the significant goals of designing affinity-based sensors is to maximize the association and minimize the disassociation of target biomolecules. As a result, saturation may occur in this kind of sensors and they will not be able to provide the user with the information about the changes in the concentration levels of the analytes at different times. However, on the other hand, the catalytic sensors are able to detect and record the fluctuation in the concentration of target biomolecules over time [67].

2.4.2. Antibodies

The receptors used in affinity-based biosensors are mostly composed of nucleic acids, immunoglobulin or antibodies, oligopeptides, aptamers, and *etc.* Among these receptors, antibodies are being used in a vast majority of biosensors. Antibody (Ab) or immunoglobulin (Ig) is a large Y-shaped protein which is created by a group of white blood cells (B cells) and then released into the blood-flow system of body. Each antibody has a unique target known as an antigen and as Figure 2.18 shows, antigen binding sites in the antibody allows the binding to the target molecule. The immune system of body recruits the antibodies to recognize and neutralize the foreign antigens like viruses and bacteria in the blood flow.

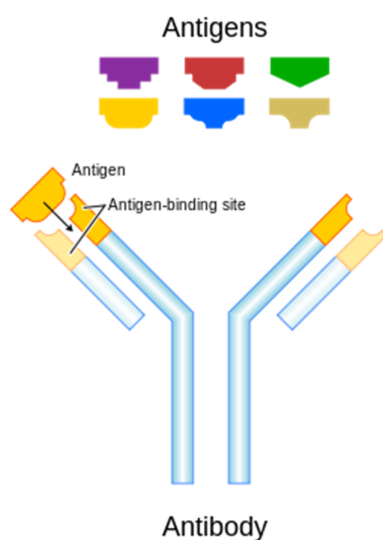


Figure 2.18. An antibody and its binding sites illustration [68].

Having the ability to combine with specific analytes, antibodies are being employed extensively as the recognition parts in biosensors to detect, identify and quantify the target biomolecules. The devices investigating and specifying the bio-molecular combination of antibodies to antigens in the aqueous media are known as immunoassays and immunosensors in the solid-state.

2.4.3. Immunosensors

Categorized as affinity antibody-based biosensors, immunosensors are miniaturized devices to identify their target analytes by means of antibody-antigen interactions in real-time fashion. The very basic operation principle of immunosensors is the coupling of immunochemical events occurring in the antibody-antigen combination to the transducer section of the sensor. The transducing methods vary from electrochemical to piezoelectric and optical. Immunosensors inherit their high sensitivity and selectivity from the recognition part (Antibodies). As reported in the literature, in the last decade, the development of immunosensors for the clinical applications [69], food analyses [70], process control [71] and environmental monitoring [72] have been increased. There are a lot of examples available in the literature at the time being, for instance, immunosensors have been reported to be used in detection of the remaining pesticides in the food products

[73]. In another work in the field of clinical diagnosis, authors have investigated immunosensors to determine the tumor markers [74].

Despite the enormous amount of research over the immunosensors, there is still a necessity to develop more realistic and commercial systems operating with elevated selectivity, sensitivity, reversibility and reliability levels [75]. One of the most practical methods to fill the gap between the theory and reality in the field of bio-sensing and immunosensors, is to investigate the production of receptors and target analytes (antibodies and antigens) on the sensor surface. So, a key step to optimize the analytical performance of immunosensors would be to study and enhance the immobilization strategies for the antibodies-antigens in the immunosensors transducers or support matrices. In the next section, the biomolecules conventional and modern immobilization methodologies will be explored and discussed.

2.4.4. Biomolecules immobilization methods

A wide range of immobilization processes are used to attach biomolecules like proteins and enzymes to different substrates. The quality of a specific immobilization method is decided by factors like immobilization surface and protein properties, buffer constituent and assay performance metrics (*e.g.* sensitivity, selectivity, reliability and reusability) [76]. These methods fall into three major classes which are described in the following.

Physical adsorption:

Physisorption is known as a very convenient and simple method to introduce receptors onto the transducers surfaces. It is based on the physical and intermolecular interactions. These interactions can be formed through the van-der Waals, hydrophobic or electrostatic forces (Figure 2.19). The main disadvantage of this method is that the method is generally weak and after being immobilized over the surface of sensor, the antibodies stand in various orientations which are depicted in Figure 2.20. There are three potential orientations for antibodies: Fully active, which is the desirable case, partially active and

inactive. It is essential for an antibody to be in active orientation to be able to bind with its corresponding antigen.

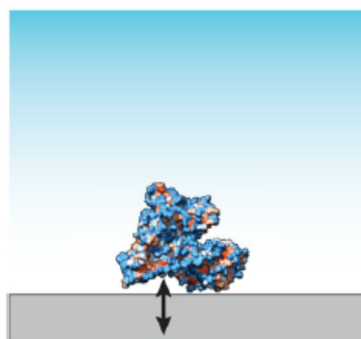


Figure 2.19. Intermolecular interactions illustration [76].

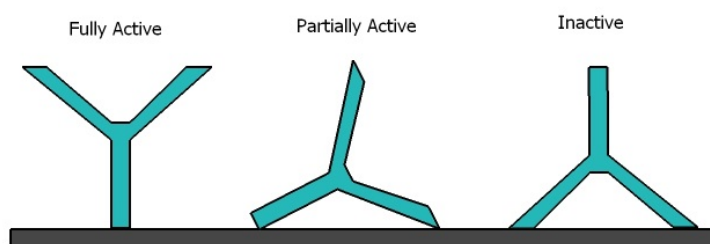


Figure 2.20. The possible orientation of antibodies after immobilization.

Bio-affinity attachment:

In this particular method, the recognition elements attach to the transducer by formation of bio-affinity interactions between the antibodies functional groups and the surface (Figure 2.21). The bio-specific adsorption method yields highly stable and strong and oriented immobilized proteins, minimized protein leakage and optimized accessibility to the binding partner [77, 78]. Furthermore, the bio-affinity interactions can be reversed by using treatment methods (chemical, thermal and *etc.*). As a result, this method provides high bio-molecular activity, reduced non-specific adsorption and better stability. Generally, the bio-affinity interactions are accompanied by physisorption or covalent bonding mechanisms by using a reagent as an intermediate binding molecule between surface and target proteins. Streptavidin-biotin interaction in this project is an example of this sort of mixed interactions, which will be investigated in this thesis later.

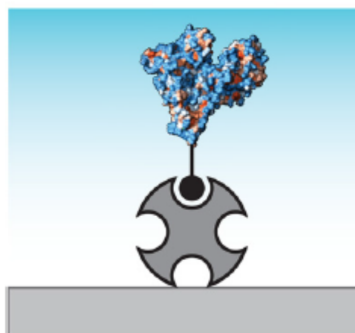


Figure 2.21. Bio-affinity interaction illustration [76].

Covalent bonding:

In this method, the immobilization substrate is activated with a reactive agent and then the activated surface interacts with the proteins and form a reversible covalent bond (Figure 2.22). Another approach to achieve a covalent bond is to use biological spacers. The spacer's one end is linked to the activated substrate and the other end is free to covalently bind to the proteins (Figure 2.23). The free standing spacers which have not reacted with proteins can be blocked by adding blocking agents to the solution. The disadvantages of this method are the reduced protein activity, complicated chemistry, and slow binding process. As an example, Wittman *et al.*, has reported immobilization of 2,4-dichlorophenoxyacetic acid on the surface of amino-silanized optical fibers, using the covalent attachment method [79].

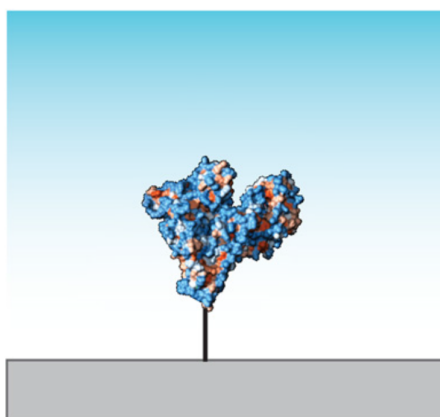


Figure 2.22. The covalent bond illustration [76].

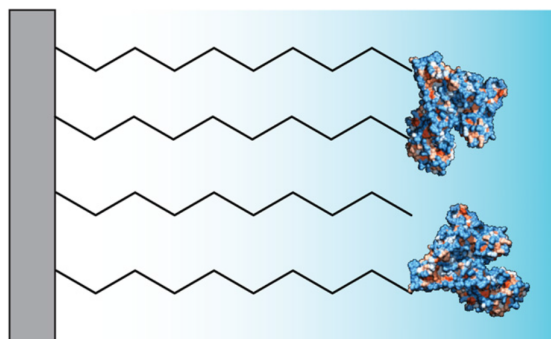


Figure 2.23. Illustration of using biological spacers [76].

2.4.5. Combined Immobilization process

As a matter of fact, each of mentioned immobilization protocols in the previous section have their own disadvantages and advantage putting limitations when used as the only immobilization process in a biosensors. Recently, a considerable number of studies have been aiming for combining the immobilization methods to sum up their advantages and attain better protein activity, more control for protein orientation, less steric hindrance, more protein patterning density, and less nonspecific protein adsorption [80-82].

Each possible combination has been reported by different groups. Physisorption-covalent bond has been investigated by Yakovleva *et al.* [80]. Eteshola and Leckband have studied the covalent-bioaffinity immobilization method and compared the passive adsorption of a capture antibody (rabbit antisheep IgM) to a PDMS microchannel with site-specific immobilization of the capture antibody using protein A [81]. The physisorption-bioaffinity combination has been exploited by Schult *et al.* to probe the hydrophobicity of PMMA to physisorbed neutravidin, and then immobilize a Biotinylated monoclonal capture antibody [82]. Finally, Dodge *et al.* has studied the mixture of three methods by creating a hydrophobic monolayer in a wet-etched microchannel over a glass surface, which in its own nature is known to be hydrophilic. Then, the protein A was physisorbed to the hydrophobic activated surface followed by attachment of fluorescently labeled rabbit IgG as a spacer. The use of this spacer provides a better condition for the

antibody in terms of activity and orientation resulting in more effective antigen capture, which occurs by bioaffinity coupling [82]. Also, there is another report in which authors have attempted to immobilize antibodies onto inert plastic surfaces by combining all of the immobilization methods [84]. Figure 2.24 depicts the process of activating the surface, adding the spacer and bonding of antibodies by electrostatic reaction and the bioaffinity reaction between the antibody and target analyte.

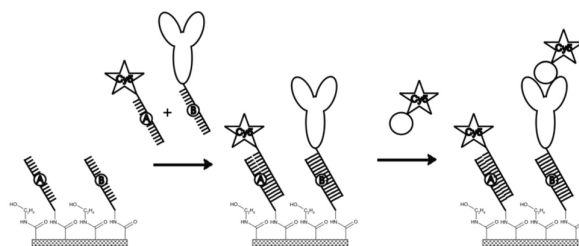


Figure 2.24. Illustration of combined immobilization methods to entrap biomolecules over a plastic surface [84].

2.4.6. Immunoassays

In this section, a summarized and short definition will be given about the immunoassays. This technology which is based on bio-sensing, exploits the sensitivity and selectivity of biomolecules' interactions to recognize, identify and quantify the target analytes. They are used in various fields such as medical-diagnostics, proteomics, pharmaceutical research and biological research to sense biological analytes. Basically, the immunoassays are divided into two main categories: heterogeneous and homogeneous. In the heterogeneous immunoassays, antibodies or in some cases antigens are immobilized over a solid support or the transducer surface and the interaction with the counter parts of biomolecules occur at the boundary layer. However, in the homogeneous immunoassays, two molecules interact with each other in a solution and the induced physical or chemical change from antibody-antigen interactions can be detected. In addition, the immunoassays can perform in two different modes: competitive and non-competitive. In the competitive mode, the target analytes compete with labeled-antigens to bind with a specific and limited number of antibodies. In this mode, the signal decreases as the concentration of the antigens increases. In the non-competitive mode, the antigens interact with the antibodies and the concentration of the targets is detected after binding of a second set of labeled

antibodies to the antigens. As a result, the output signal's amplitude will be proportional to the antigen's concentration. This sort of immunoassays are called “sandwich immunoassays”.

The bio-affinity based bio-sensors can be considered to behave like a heterogeneous immunoassay. The main problem with the heterogeneous mode immunoassays is their labor-intensive preparation steps and the long period of time essential for the incubation and detection of sample analytes because of the biomolecules' diffusion in long distances to reach the boundary layer [85]. One of the mostly investigated solution to the encountered problems with the immunoassays is to miniaturize the structure or incorporate microfluidic systems within the devices. In the following section, more details and explanations will be given for this case.

2.5. Microfluidics and Bio-sensing

Recently emerged and an intriguing subject for research groups and scientists, the microfluidic channel systems have attracted very much attention. The microfluidics technology, which is still in its infancy after passing 15 years from its invention, is proved to enhance the performance of immunoassays and immunosensors in many terms [86, 87]. In general, this new and powerful tool seeks to improve the analytical performance of immunosensors by decreasing the consumption of the agents in the result of flowing liquid into the micron-scale fluidic networks, reducing the diffusion time and solution volume; thus increasing reliability and sensitivity of system. Additionally, the automation and multiple sample analysis can be achieved by miniaturizing the immunoassays systems with microfluidic channels. The methods and smart protocols for optimizing and controlling the protein and biomolecules' immobilization inside the microchannels are vastly reviewed and discussed in a recent review by Dohyun Kim and Amy E. Herr [76]. Figure 2.25 from Yun Liu *et al.* [88], gives a schematic of a PMMA microfluidic channel incorporated into a three electrode detection system in order to detect low-level disease biomarkers.

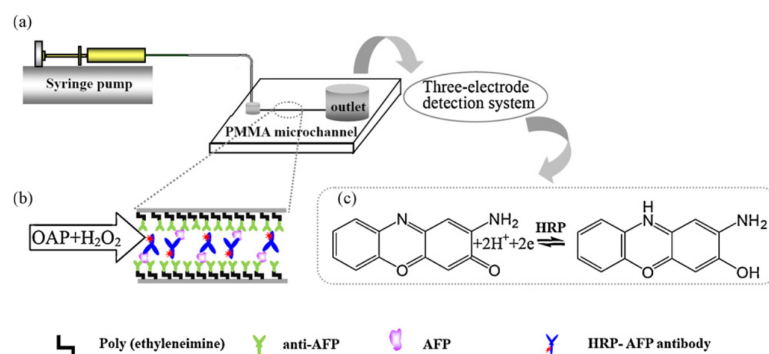


Figure 2.25. (a): The syringe used to pump solution containing biomolecules into channel (b): enzyme-linked immunosorbent assay in the PMMA microchannel (c): Catalytic reaction occurring in the channel [88].

The interesting and remarkable properties of microfluidic channels, which are studied in review by Adam Bange, H. Brian Halsall and William R. Heineman in 2005 [87], have made them a prosperous technology for immunoassays and immunosensors applications. Offering promising features to improve biosensors operation and performance, micro-scale channel networks can be investigated in the future works and studies related to the topic of this thesis. The most outstanding enhancements that they provide to the analytical immunoassays are explained below:

- Decreased amount of necessary biomolecules and reagents which decreases the total cost of the experiments.
- Less analysis time due to the shorter diffusion distance for antigens or antibodies. This ability makes it possible to monitor analytes in a real-time and continuous manner with a relatively high sensitivity, selectivity and reliability.
- The microfluidics ability to control and automate the liquid flow in the channels, can drastically decrease the amount of human error which is a big source of loss of precision in bio-sensing processes. This automation can be achieved by softwares controlling the valves, pumps, mixers and other elements in microchannels.
- Having larger surface area to volume ratio and smaller fluidic volume, integration of microfluidics into immunoassay systems yields rapid diagnostics, increases the speed at which the results can be obtained and considerably decreases the external

essential power for the system to operate. This helps to build portable passive devices needing little energy to run the analyses.

Promising significant and prominent advantages, microfluidics have been reported in the recent years in a bulk number of studies and still are considered as a fertile field for more research and investigation. Several works have been done to exploit rapid, point-of-care and sensitive characteristics of the microfluidic integrated immunoassays in the detection of disease biomarkers such C-reactive protein [89-91] and HIV-specific antibodies [92]. They have also been utilized for medical diagnostics purpose to detect proteins and antibodies like pregnancy hormone in human serum [76], human Thrombin [93], Cholesterol Modulated Antibody [94] and anti-DNP antibodies [95]. Some studies report these devices to be used in environmental monitoring as well [96, 97]. They also have been used recently in sample-in-answer-out optimized genetic analyses [98, 99].

As an important issue, the design and implementing of microchannels face a number of challenges that should be overcome. Micro-dimension channels can be utilized in both heterogeneous and homogeneous based immunoassays. As a consequence, there are a number of common challenges. A list of challenges and explanations about why they are important are given below [100-103]:

- The material from which the microchannels are built is a key factor in designing them because of the large surface area to volume ratio that they present.
- Specific surface modification methods are essential to prevent adhesion of key biomolecules onto the microchannels surfaces.
- The microfluidic system design is considered as an eminent challenge to achieve the best possible performance of channels to deliver and drive the solutions
- An effective strategy must be developed to introduce, and move the sample flow in the channel to deliver the biomolecules either to a solid support or to a different solution containing target analytes.

- Smart and suitable immobilization protocols are necessary for attaching the molecules to a solid support in the case of heterogeneous immunoassays.
- The most compatible transducing technique with the micron dimension should be implemented in the device to detect the antibody-antigen bindings in a much more efficient manner.

One of the major strategies to enhance the surface-based immunoassays system operation, is the analyte delivery and washing method. Although investigating the mentioned challenges in the above can improve the performance of immunoassays, this method is discussed mostly in details herewith, because of possible integration with the subject of this project in the future to reach and built a more sensitive and reliable device.

As a matter of fact, implementing original and novel analyte delivery methods can increase the sensitivity, decrease the analysis time and improve the detection limits as well. Although the micron sized microchannels reduce the diffusion distance for the analytes considerably, their traveling toward the boundary layer still remains transport depended at low-concentrations. A simple way to overcome this issue is to reduce the dimensions further, but this will add to the fluidic resistance which is not desired.

An innovative flow-confinement method to circumvent this problem is reported by Hofman *et al.* [99]. In this method which is designed for rapid delivery of biomolecules to the recognition surface in low sample concentrations, a water or sample medium flow is joined with a perpendicular injection to confine the targets into a thin layer above the sensors surface. Figure 2.26 clearly illustrate this concept.

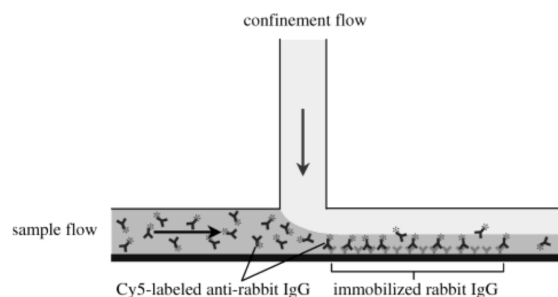


Figure 2.26. Illustration of confinement concept. The confinement flow is injected to confine the sample flow to a thin layer over the immobilized IgG [99].

Microdiluators networks have been reported in a study as mixing elements to serially dilute two streams which can be sample solution and buffer. The reported network which is shown in Figure 2.27 is capable of analyzing different antibodies in a same time [100].

The diffusion, is the formation of a depletion layer on the sensor surface. The reason for this phenomena is the binding of targets with antibodies which replenish the target molecules adjacent to the surface. Golden *et al.* has reported a novel way to avoid the formation of depletion layer and maximize the target transport from the bulk solution to the substrate, by patterning grooves over the microfluidic channels [101]. The embossed grooves, which are illustrated in Figure 2.27, would help to mix the solution, enhancing the target delivery and increasing the sensitivity.

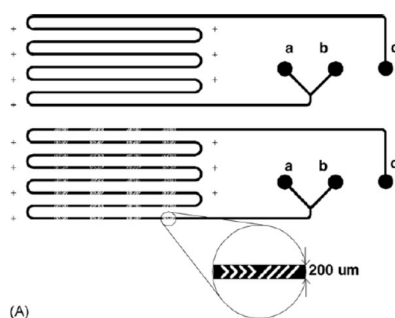


Figure 2.27. The microfluidic channels and the patterned grooves on their surface [101].

The earlier works, reporting to solve this problem with mass transport idea [100, 102] intensify the complexity of the device by working with two fluids simultaneously and also needing high amount of buffer solution to be injected into the system continuously.

In addition to the investigated pathways to improve performance of surface based immunoassays, active forces as another strategic tool, are proved to help to achieve high levels of sensitivity and specificity in biosensor systems. A creative technique named as fluid force discrimination (FFD) was reported by Mulvaney *et al.* [103] to remove non-specific absorptions, obtain multiplexed, unamplified, femto-molar detection limits and achieve under 10 minutes analysis time. The FFD mechanism is such that the captured target molecules are labeled with microbeads and microfluidic forces are applied using a

controlled laminar flow, so that all the non-specified bindings are washed away. The density of the reaming beads, which can be detected by opto-counting or magnetoelectric detection, will be proportional to the analyte concentration. Figure 2.28, shows a non-specific absorption on left and a specific one happening at the same time on the right of the picture, while both of them are labeled with microbeads.

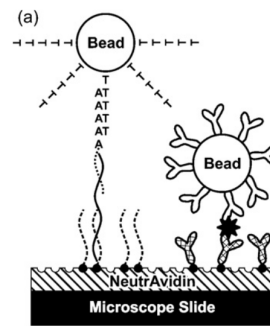


Figure 2.28. Non-specific and specific absorption with microbeads attached to them [103].

There are also several other configurations of microfluidic immunoassays that are discussed widely with more details in a review published in 2010 [79]. The micro-channel networks based biosensing systems show great potential to obtain commercially-approved high sensitivity, reliability, point-of-care diagnostics, fast and precise analysis, low-power consumption, better integrability and portability, less non-specific absorption and several other properties. However, the currently studied systems in laboratories by various research groups do not fulfill the conditions and requirements for the vast practical and commercial applications of them. The on-going and developing technologies and methodologies to improve the antibody-antigen binding promise a bright and flourishing future for them. Several design considerations and challenges were explained shortly and strategies to circumvent one of the major obstacles to reach a real well-done device were mentioned. Further studies over the discussed techniques to improve the mass transport from bulk solution to the surface, reduce the diffusion distance and time and eliminate non-specific absorption in an antibody-antigen bound based biosensor, are considered to be a novel subject for the future studies.

2.6. OFETs as biosensors

Because of their enormous advantages and their potential to be used in disposable and cheap sensing devices, organic materials have been studied and implemented as active materials in biological and chemical sensors [104-108]. As a result, organic field-effect transistors which incorporate organic materials into their structure and demonstrate a stable and reliable behavior when are exposed to the organic materials containing biomolecules, can be utilized as both bio-detector and transducer sections of a biosensor. OFETs employing electrolytes at their gate regions and polymers as their active layers, have potential advantages such as; ease of fabrication using low-cost, low-temperature processes, ability to improve their physical and chemical sensitivity and selectivity by changing their molecular structures, possibility of introducing recognition elements into their active regions or bulk material plus ability to incorporate new materials to their electrolyte layers. These advantages make electrolyte gated OFETs a suitable choice for the matter of chemical or biological sensing [3-7].

Electrolyte gated OFETs have shown stable and reliable responses with aqueous media, which allow the soluble biological analytes to be introduced to the devices [9]. Furthermore, signal amplification nature of a transistor makes an organic field-effect transistor to function as a good transducer element. Some studies in the literature have reported OFETs to be utilized as sensors to measure humidity of the atmosphere around them [9, 10], airborne explosive vapors like DNT and TNT [11, 12], and to sense alcohol [13].

A water-gated organic field-effect transistor (WGOFET) which shows sub-one-volt operation has been introduced and investigated by Kergorat *et al.* [18]. Pure deionized water as the gate electrolyte eliminates the electrochemical switching from the field-effect modulation and this advantage was used in a study to report a DNA sensor based on water-gated field-effect transistor [108].

In this particular project, we introduce a unique water-gated OFET with a simple and innovative planar gate electrode structure to be utilized in a biosensing cell. This sensor cell is composed of the mentioned transistor and a tiny plastic o-ring to simulate a

microfluidic channel system embedded over the OFET. A thin layer of polythiophene was used as the semiconductor layer and deionized water was used as the insulator material. In order to experiment the theory of the WGOFET as a biosensor, biotin-streptavidin biomolecule pairs were utilized for biological interactions.

In this particular project, we introduce a unique water-gated OFET with a simple and innovative planar structure to be utilized in a biosensing cell. This sensor cell composed of the mentioned transistor and a tiny plastic O-ring to simulate a microfluidic channel system imbedded over the OFET. A thin layer of polythiophene was used as the semiconductor layer and deionized water was used as the insulator material. In order to experiment the theory of the WGOFET as a biosensor, biotin-streptavidin biomolecule pairs were utilized to model biological interactions.

2.7. Biotin and Streptavidin: A model for protein based interactions

Having found a good number of applications in the field of biosensors, the biotin and streptavidin, are two famous biomolecules, whose interaction is known to be one of the strongest. This interaction have a high dissociation constant of about $K_d \cong 10^{-14}M$, which allows investigation of the binding in the milli or even micro molar ratios.

Biotin is a water-soluble B-vitamin whose chemical structure is shown in Figure 2.29. Streptavidin, a 52.8 kDa protein, is a tetramer biomolecule which has four different binding hosts for biotin. Figure 2.30 illustrates a streptavidin molecule structure and the binding site in each four corner. The binding of each of the monomers of the streptavidin to the biotin molecule ensures the high stability of the interaction against the disassociation and provides the strong affinity. Figure 2.31 illustrates three different situations in which streptavidin molecule bind to the biotin molecules.

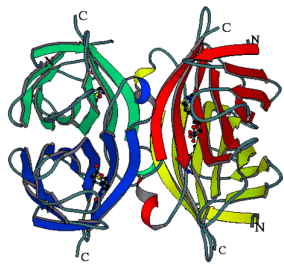


Figure 2.29. Streptavidin molecule structure [109].

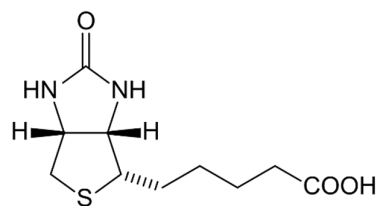


Figure 2.30. Chemical structure of biotin molecule [110].

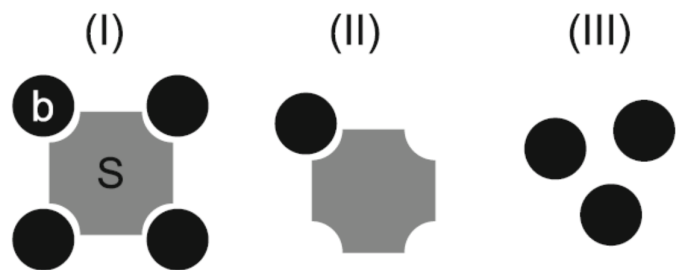


Figure 2.31. (I): Three different possible interactions of streptavidin molecule with biotin molecules. (I) Saturated: all the four heads are bind to biotin molecules (II) Half-Saturated (III) No binding occurred [111].

Showing high non-covalent affinity, the biotin-streptavidin interactions have been used in different fields like fixation of biomolecules to different substrates as well as applications in the biosensing field to model protein based bindings. For example, in a report the authors have immobilized biotin into a polymer layer which was used in a TFT whose performance as a biosensor was evaluated by the introduction of avidin molecules to its surface [112]. In a different study, again the biotin biomolecules were immobilized on a metal mesh surface with a thiol-end technique and a special sensor was utilized to detect the interactions of streptavidin molecules to the immobilized molecules [113]. In a study electrochemistry and conductivity of biotinylated electrodes have been investigated

for the detection of added avidin biomolecule [114]. In other similar reports, biotin molecules has been functionalized into the polymer layer and has been used in a direct contact with an electrode to detect the change in electrochemical response upon the addition of avidin molecule[115]. It is also incorporated into a nanotube FET device to measure the device's electrical characteristics in response to the existence of streptavidin biomolecule [116]. Also an OFET based biosensor for biotin-avidin couple detection was reported .The active layer which is a sensitive biotinylated polymer layer has been exposed to the solution containing avidin and in the result of the coupling between biotin and avidin drain current reduction has been observed [117].

As the latest work for the detection of protein bindings, Suspene *et al.* has reported a water-gated filed-effect transistor into which functionalized polythiophene is incorporated. The model proteins which are avidin, streptavidin and BSA are added to the electrolyte material (PBS). As a result of coupling, the changes in square root of I_{ds} versus the gate voltage have been measured and investigated [118].

Consequently, based on the results in these reports and their vast applications, streptavidin and biotin proteins are considered and used in this thesis to model the practical biological interactions.

2.8. WGOFET based biosensor

As the main topic of this thesis, we propose a unique water-gated OFET with a simple and innovative planar structure to be utilized in a biosensing cell. The low-temperature, inexpensive fabrication processes, and compatibility with organic materials and solvents, are of great merits of the introduced planar WGOFET. The designed biosensor cell, which is illustrated in Figure 2.32, has three major parts. First part is a thin film field-effect transistor, which is the core of the biosensor and has three main electrodes; source, drain and gate. Second part is a thin layer of functionalized polythiophene containing biotin molecules which is spin coated over the transistor to construct the semiconductor layer for the OFET. And the final part is an incubation setup, which is utilized to simulate a microfluidic channel over the active layer of the OFET and

also ensures a reliable and safe environment for the water solutions, which were used as the insulating layers in the OFET. This part simply consisted of a tiny plastic o-ring, over which a small piece of glass was put to cover the solution inside of it. This approach was taken to prevent the evaporation of solutions inside the o-ring as well.

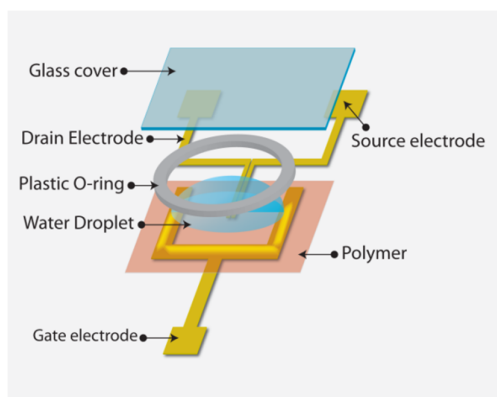


Figure 2.32. The designed sensing cell unit composed of WGOFET, polymer layer and the incubation setup

In this particular device, the biotin molecules were introduced to it by functionalizing the polythiophene polymer chains with biotin molecules. The biofriendly environment of insulator layer of the WGOFET made it possible for us to add streptavidin biomolecules to the layer and introduce them to the immobilized biotin biomolecules. After the exposure, the transistor characteristics were measured and analyzed to see if the binding of streptavidin to biotin molecules has affected OFET's electrical features. In the next chapter, we are going to explain the fabrication and preparation steps for the sensor cell and give more detail about its structure.

3. EXPERIMENTAL

3.1. Water gated OFET fabrication

The mentioned water gated OFET was fabricated over a 1.1mm thick glass wafer coated with a $50\text{ \AA}$ thick layer of chrome and a $500\text{ \AA}$ thick gold layer. After cleaning, washing and drying the wafer, it was coated with a layer of photoresist and using a UV exposure method, a single mask which is illustrated in Figure 3.1 was used to define the transistor's electrodes. The mask defined the source and drain electrodes which were separated with a $2\mu\text{m}$ gap and two larger pads connected to them to simplify the matter of getting contact to these electrodes. A gate electrode with a same large pad connected to it, surrounds the drain and source electrodes allowing induction of the field-effect to the active layer of the OFET. After defining the electrodes over the PR layer, the wafer was emerged in a gold etchant solution for 20 seconds and then the photoresist layer was removed. In the following step, the remaining chrome layer under the gold layer was etched away using a chrome wet etching solution. A real picture of a fabricated transistor over glass surface is given in Figure 3.2 to give more understanding of the planar field effect transistor.

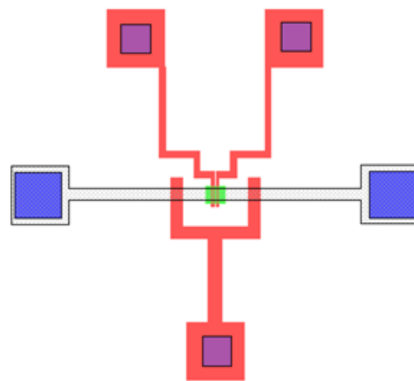


Figure 3.1. The designed mask describing the structure of WGOFET.

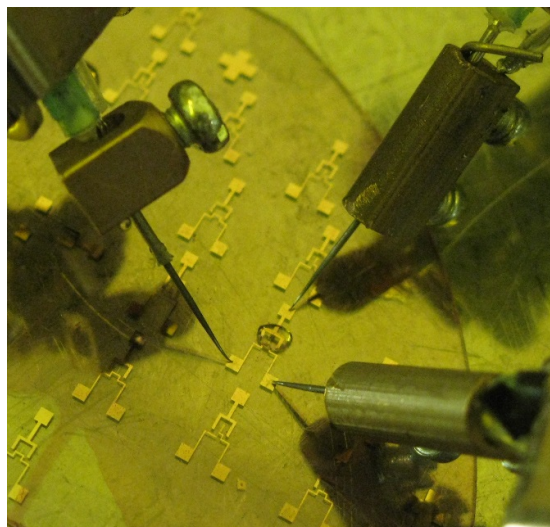


Figure 3.2. Fabricated electrodes with polymer film and electrolyte droplet.

Figure 3.3 illustrates the taken steps for the preparation of sensor cell. Gate, drain and source electrodes were patterned with lithography and built over the glass wafer in the first step. The transistor length and width were designed to be $2\mu\text{m}$ and $500\mu\text{m}$. In the second step the biotinylated-polymer solution was prepared in chlorobenzene solvent with a ratio of 5 mgml^{-1} and then spin coated over the wafer. After spin coating the polymer, a sharp pin was utilized to mechanically scratch and clean-off some of the coated polymer on the gate electrode in order to make sure that the electrolyte layer will maintain a contact with gate electrode as well as active layer. In this structure, the source and drain electrodes do not have any electrical connection to the water solution since they are covered with the polymer film. In the following steps, a small plastic o-ring was placed over the active region of transistor and then the DI droplet was put into the o-ring using a micro-syringe. Finally, a piece of glass was put over the o-ring to confine the droplet and achieve a good isolated environment for the water solution.

This step was done to prevent the droplet from evaporating in the time of conducting the experiment and at the same time creates a system similar to that of microfluidic channels. This approach can be useful to evaluate the future potential of integrating this device with real microfluidic channels to introduce target biomolecules.

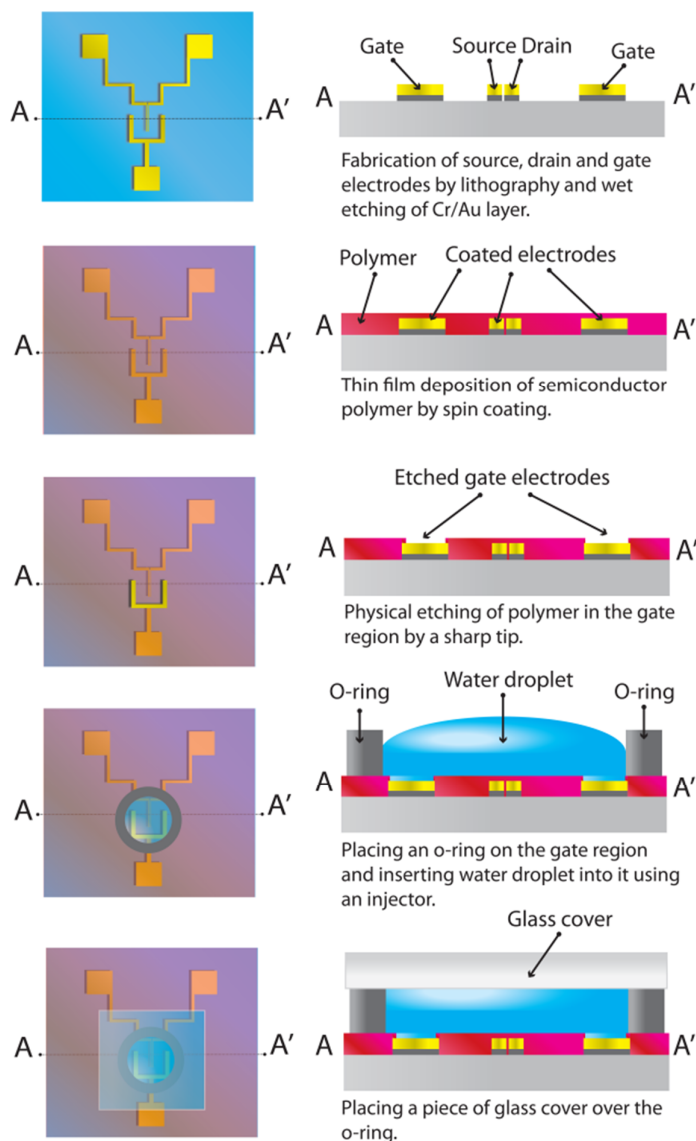


Figure 3.3. The illustration of the fabrication and preparation steps for the biosensor cell.

In the next section, the procedure for functionalizing the polythiophene with biotin biomolecules is described. This process was conducted by K. Merve Türksöy at the Chemistry Department of Bogazici University.

3.2. Synthesis of biotinylated regioregular polythiophene

The steps for the synthesis is shown by Figure 3.4 and the numbers written in the parentheses below point to the steps illustrated in this figure. Biotin azide (6) was prepared according to the literature from biotin succinimide (3) [119]. Similarly, the synthesis of regioregularly designed poly(3-butynylthiophene-co-3-hexylthiophene) (P3TBT-co-P3HT, 5) was reported by Venkataraman *et al.* [120].

According to the procedure, commercially available thiophene-3-methanol was chlorinated by HCl. Being degradable in time, 3-chloromethyl-thiophene was converted to triisopropyl-(4-thiophen-3-yl-but-1-ynyl)-silane as soon as possible with the reaction of 1-triisopropyl- 1-propyne (1-TIPS-1-propyne) in the presence of n-BuLi. To create the monomers 3.5 equiv of NBS was used to brominate the product. After that, resulting [4-(2,5-dibromo-thiophen-3-yl)-but-1-ynyl]-triisopropyl-silane was deprotected by TBAF and poly(3-butynylthiophene-co-3-hexylthiophene) (P3TBT-co-P3HT, 5) was obtained. The ^1H -NMR spectrum of the material agreed with that reported (alkynyl peak at δ 1.97 ppm) and GPC indicated $M_n = 15000$, PDI = 1.2.

Compound (5) (60 mg, 0,004 mmol) in 1 mL THF was added to the solution of Cu(I)Br (1.7 mg, 0.012 mmol) in dry THF (0.2 mL) and purged. To the mixture was added the solutions of biotin azide (13 mg, 0.04 mmol) in 1 mL dry DMF and PMDTA (*N,N,N',N',N''*-pentamethyldiethylenetriamine) (1 mg, 0.006 mmol) in 0.5 mL dry DMF. Resulting solution was stirred at 50°C for 2 days. Reaction mixture was then precipitated with MeOH (50 mL) and collected through filtration as a purple solid (56 mg, $M_n = 16000$, PDI = 1.3). Finally, the biotinylated-P3HT was synthesized with the final step shown in Figure 3.4.

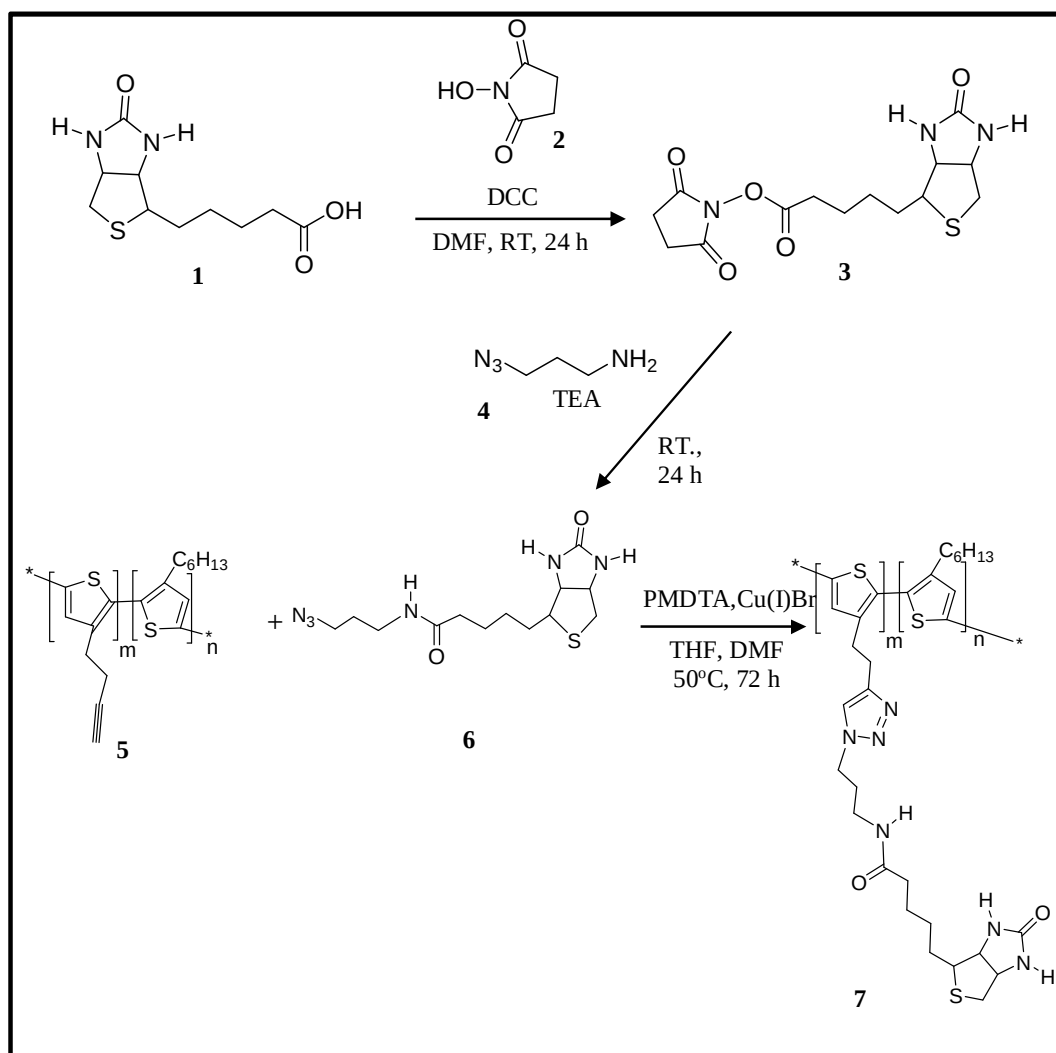


Figure 3.4. The biotinylated P3HT synthesis steps.

4. RESULTS AND DISCUSSION

4.1. Electrical measurement of the WGOFET

The fabricated transistors' output electrical characteristics were extracted and recorded using a Keithley 4200-SCS semiconductor analyzer shown in Figure 4.1. Biotinylated P3HT was spin coated over the device and pure DI water was placed inside the o-ring and covered by the cover glass as the electrolyte material. Connections from the analyzer to the device were directly made to the gate, drain and source electrodes and the electrical transfer characteristics of the device were measured.



Figure 4.1. Keithley 4200-SCS semiconductor analyzer station.

Figure 4.2 shows a picture of fabricated device and the connections. To evaluate the performance of the setup that we had arranged, after incubating a DI water droplet over the active region, the response of transistor based on both commercial-P3HT and biotinlayted-P3HT were extracted and compared to make sure that the device will function in a similar way that it functioned with a commercial P3HT as the semiconductor.

After conducting the measurements for both polymers, the transfer curves were observed to be much like each other. Figure 4.3 and 4.5 shows $I_{ds} - V_{ds}$ and $I_{ds} - V_{gs}$ transfer curves for a commercial-P3HT based OFET and Figures 4.4 and 4.6 demonstrate $I_{ds} - V_{ds}$ and $I_{ds} - V_{gs}$ curves for a biotined-P3HT based device. It was understood from

the results that the biotined-P3HT based OFET operates like a normal field-effect transistor, which has a linear region at low drain voltages and saturation region for the higher drain voltages. Some other electrical features of the water-gated OFET like charge carrier mobility (μ) in the saturation region were calculated using the formula given below:

$$I_D = \left(\frac{W}{2L}\right)\mu C(V_{gs} - V_{th})^2 \quad (4.1)$$

In this equation, C stands for the capacitance per unit area of water-biotinylated P3HT double-layer and W and L represent the width and length of active area of the transistor. In our calculation we have assumed the C to be roughly about $3 \mu F cm^{-2}$ as it was assumed in a previous work [121]. To calculate the transistor threshold voltages (V_{th}), $I_{ds} - V_{gs}$ curves were used. The calculated values were used in a program written in MATLAB to extrapolate the transfer curves. The electrical parameters were calculated for both commercial-P3HT and biotined-P3HT based water gated OFETs. The values are listed in the Table 4.1. The table also contains information about other measured electrical characteristics such as I_{DSAT} and I_{on}/I_{off} ratio.

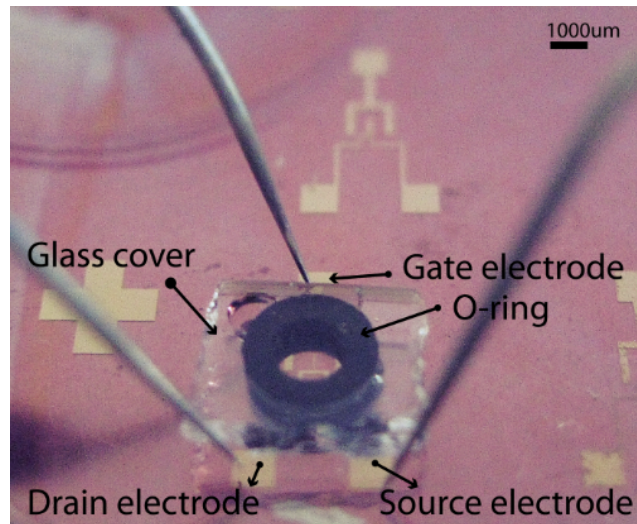


Figure 4.2. The fabricated and prepared biosensor cell under measurement.

Table 4.1. Electrical characteristics of commercial-P3HT and biotinylated P3HT based DI water-gated OFETs.

Electrical features	$I_{DSAT}(nA)$	$V_{th}(V)$	$\mu(cm^{-2}V^{-1}s^{-1}) \times 10^{-3}$	I_{on}/I_{off}
Commerical-P3HT	-300	-0.33	4.5	125
Biotined-P3HT	-268	-0.2	2.8	220

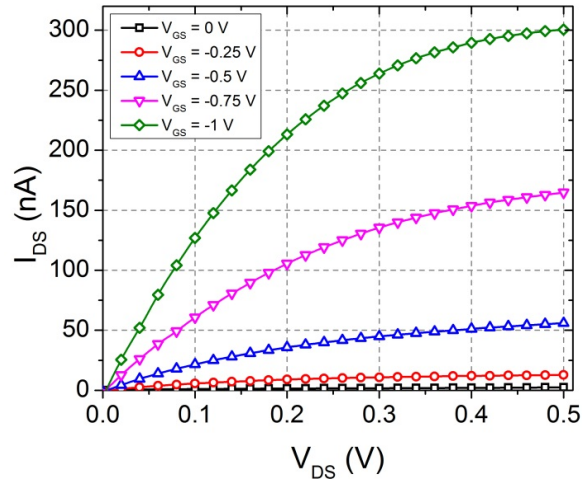


Figure 4.3. The $I_{ds} - V_{ds}$ transfer curve for commercial-P3HT based WGOFET.

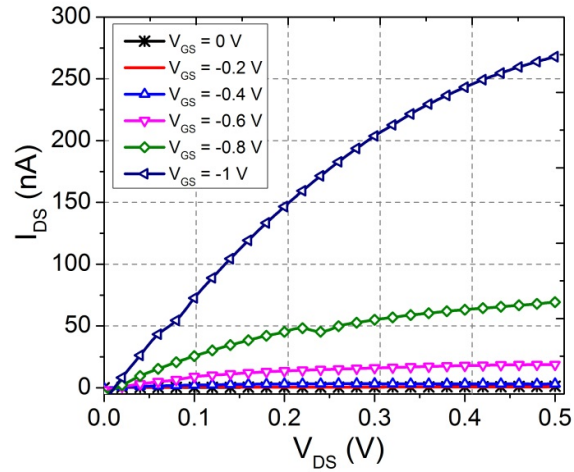


Figure 4.4. The $I_{ds} - V_{ds}$ transfer curve for biotinlayted-P3HT based WGOFET.

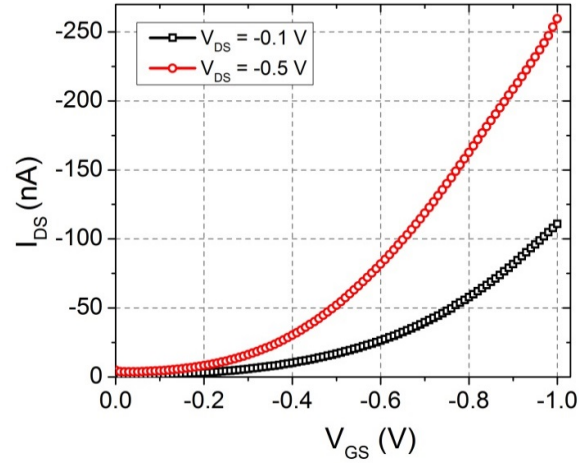


Figure 4.5. The $I_{ds} - V_{gs}$ curve transfer for commercial-P3HT based WGOFET.

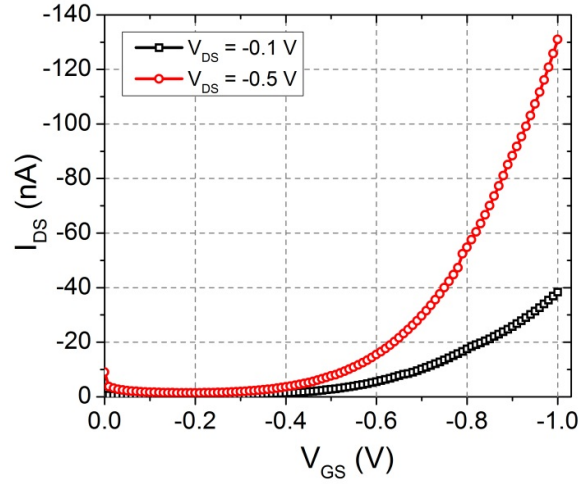


Figure 4.6. The $I_{ds} - V_{gs}$ curve transfer for biotinylated-P3HT based WGOFET.

4.2. Results for Biotin-Streptavidin interactions

To test and validate our proposed hypothesis about the potential of WGOFETs to be able to sense the protein bindings and respond to them, after immobilizing the biotin molecule by functionalizing it with P3HT and spin coating it over the device surface, streptavidin molecules were introduced to the sensor substrate in three different concentrations dissolved in DI water: 0.4 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$ and 2.5 $\mu\text{g/mL}$.

The experiments were conducted by extracting transistor transfer curves at certain intervals after putting a droplet of desired solution into the o-ring. The tests were conducted for 30 minutes and the data were saved in the analyzer. After taking a reference measurement with pure DI water, droplets of streptavidin solutions starting from the less concentrated one, were put inside the o-ring and the transfer curves were measured.

During the experiments $I_{ds} - V_{ds}$ and $I_{ds} - V_{gs}$ curves were extracted and saved for further analysis. It was predicted that exposure of streptavidin molecules to the biotinylated surface of OFET would induce an impact over its electrical characteristics. Measured data showed that the probable interaction between streptavidin and biotin molecules have caused the on current of the OFET to decline as the concentration of streptavidin increases.

The same experiment is also repeated with a commercial P3HT film instead of biotinylated one. The results from this experiment showed that the on current was also changing with streptavidin concentration, but the change did not follow any order.

The on current values of the WGOFET based on biotinylated P3HT for different streptavidin concentrations were extracted from the $I_{ds} - V_{ds}$ curves during the 30 minutes period of time. These data are demonstrated in Figure 4.7 compared to the data for the reference measurement for the sake of a better comprehension of the decrease in the transistor's on-current. Figure 4.8 summarizes the results extracted from the experiment done with the commercial P3HT based WGOFET.

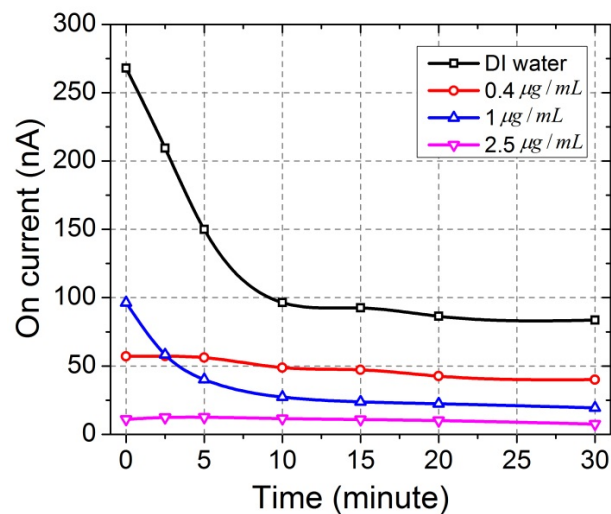


Figure 4.7. The on current values for the DI water and three different streptavidin solutions for a biotinylated P3HT based WGOFET.

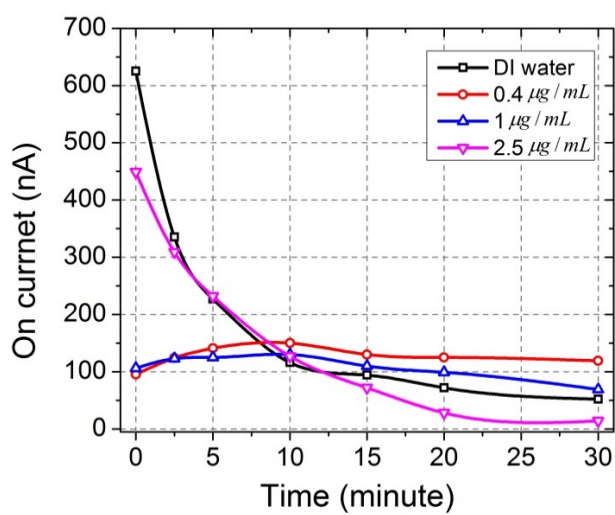


Figure 4.8. The on current values for the DI water and three different streptavidin solutions for a commercial P3HT based WGOFET.

4.3. Discussion

In order to present the on-current reduction with streptavidin concentration in a more understandable way and compare it to the one extracted from the commercial P3HT based device, the values of the on currents after 30 minutes for different streptavidin solutions were put in a single graph presented in Figure 4.9. The red curve corresponds to the on current of biotinylated P3HT based device and black curve represents the same data for the commercial based device.

It is concluded that the on-current of the transistor based on biotinylated P3HT decrease in a predictable manner, which can be approximated to a logarithmic behavior. On the other hand, the on current value varies unpredictably for the commercial P3HT based device. This supports the prediction that the ordered change of on-current in the biotinylated devices is due to the biotin-streptavidin interactions.

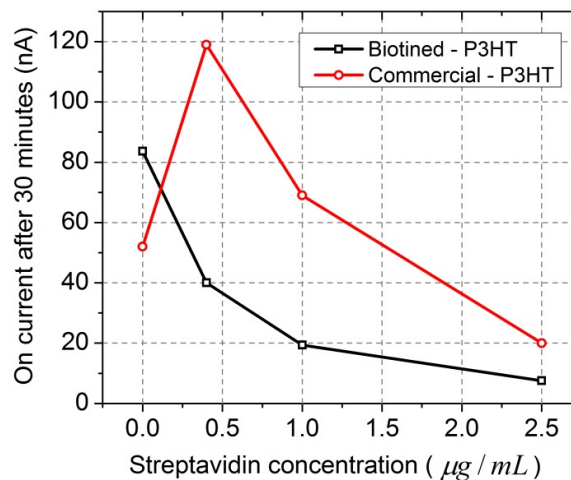


Figure 4.9. Comparison of on-current values for both biotinylated P3HT and commercial P3HT based devices after 30 minutes for various streptavidin concentrations.

In order to seek for further validation of our statement about the effect of biotin-streptavidin interactions on the OFET characteristics, the number of streptavidin and biotin molecules were roughly measured and estimated to see if the introduced amount of streptavidin molecules were saturating the biotin molecules during the experiment.

To do the calculation, the volume of the O-ring was calculated and the numbers of streptavidin molecules based on the solution ratio and the streptavidin molecular weight were estimated. Next, using the information from the synthesized P3HT and its molecular weight and ratio of active region to the whole coated area, the number of biotin molecules presented in the environment exposed to the streptavidin molecules were calculated.

The calculation details for each of the streptavidin solutions are given below:

- For the solution of 0.0004 mg/mL:

Molecular weight of Streptavidin = 52.8 kDa = 52.8k g/mol

O – ring volume = 103 mm³ = 0.103 mL

→ Amount of streptavidin in o – ring = 4.12×10^{-5} mg

1 mol = 6.022×10^{23} molecule

→ Numer of streptavidin molecules in o – ring

$$= 7.8 \times 10^{-13} \times 6.022 \times 10^{23} = 46.97 \times 10^{10}$$

- For the solution of 0.001 mg/mL:

Numer of streptavidin molecules in o – ring = 117.43×10^{10}

- For the solution of 0.0025 mg/mL:

Numer of streptavidin molecules in o – ring = 293.6×10^{10}

And the number of biotinylated P3HT chains were estimated:

Number of biotined-P3HT in active region = 109.8×10^{10}

These estimated numbers state that at least the highest two ratio solutions may have saturated the sensor. However, it is believed that a major concentration of streptavidin has been absorbed by the surfaces of o-ring and glass cover. Hence, our sensors were not saturated by the tested concentrations of streptavidin.

Another major factor affecting our device is the time needed for the streptavidin molecules to diffuse through the solution to reach the biotin molecules. Diffusion time in a two

dimensional environment for the streptavidin molecules was calculated to consider its effect, too.

In order to be able to calculate the diffusion time for streptavidin, its diffusion coefficient must be known. This value has reported to be around $D = 40 \times 10^{-6} \text{ mm}^2/\text{s}$ [122] and $D = 49 \times 10^{-6} \text{ mm}^2/\text{s}$ [123]. Simple diffusion equation can be used to extract the time needed for the proteins to diffuse along a known distance which in our case is the height of the o-ring($x = 0.5 \text{ mm}$).

Simple diffusion equation:

$$t_d = \frac{x_d^2}{qD} \quad (4.2)$$

In this equation q is the dimensionality constant and is equal to 2 in our conditions. By putting the values in the equation and assuming the diffusion constant to be equal to $45 \times 10^{-6} \text{ mm}^2/\text{s}$, the diffusion time can be calculated to be equal to 46 minutes which is more than what we had allowed the streptavidin biomolecules to diffuse and interact with biotin molecules.

Based on these calculations it can be concluded that even though the amount of streptavidin molecules in the environment may saturate the sensor, as the diffusion time implies, the experiments' time was not long enough to ensure that all of the streptavidin molecules have reached to the sensor substrate. So, this conclusion may uphold the proposition that streptavidin-biotin interactions has induced changes over the electrical characteristics of the WGOFET and as the concentration of the streptavidin molecules has increased, the reduction in the value of that specific electrical characteristic, which in our case is the on-current of the transistor after 30 minutes, has followed a predictable order.

The previous figures showing transistor results are from individual devices. However, the same experiments were repeated with two different batches of biotinylated P3HT. In each batch, five different OFET devices are tested. The same trends were observed in all of these experiments. Considerable differences in the performance of every OFET device are due to the geometric factors such as the changing thickness and

roughness of the polymer film, and alignment errors of the o-ring with respect to electrodes. Therefore, electrical characteristics of devices vary from device to device and batch to batch. That is why there are no error bars in the figures.

The lack of passivation on non-active device surfaces may have resulted in non-specific interactions and adsorptions inside the o-ring which can affect the transistor's response. Introduction of ions to WGOFET structure can induce similar changes in the electrical characteristics of the device as seen with the interaction of streptavidin with the biotinylated OFET.

Consequently, more experiments and works are needed to understand the effect of streptavidin binding on the electrical characteristics of the biotinylated OFET. Control experiments are needed eliminate the effects of non-specific adsorptions in the sensor. The surface of the biotinylated P3HT polymer may be treated to eliminate any non-specific interactions.

5. CONCLUSION AND FUTURE WORK

5.1. Conclusion

In this particular investigation of the water gated planar field-effect transistors, conclusions based on the obtained results will be summarized and possible future works to enhance the performance of the device and maintain a good biological sensing behavior will be discussed.

Important points and conclusions of the project are summarized below:

- A novel thin film field-effect organic transistor in which the insulator layer consists of a droplet of DI water was introduced and fabricated. By referring to the two previous studies by the Berkan Yaman [31] and Ismail Terkeşli [42] with the same device, the unique advantages of this particular sort of OFET can be listed as simplicity, ease of fabrication, being integratable to micro-fluidic channel networks, and compatibility with various water solutions as their insulator layers.
- Considering the merits of the WGOFETs, we have designed a biosensor device based on it, in which biological interactions can be detected.
- Streptavidin-biotin interaction was chosen to model protein bindings in the biosensor. Biotin was introduced to the sensor substrate by functionalizing polythiophene polymer chains with it. The biotinylated P3HT then was spin-coated over the transistor to form the semiconductor layer. Streptavidin molecules were dissolved in the pure DI water in three different ratios.
- A small plastic o-ring was utilized to exploit the WGOFET ability to be integrated with microfluidic channels. To maintain a situation similar to that of a practical microfluidic channel a tiny piece of glass was put over the o-ring to isolate the materials inside from surrounding environment.
- Pure DI water and Streptavidin droplets were inserted into the o-ring and were confined in it to ensure a stable and reliable condition for the running experiment. The

transistor output characteristics were extracted at specific times in each single experiment.

- The received data, showed an ordered reduction in the on-current of the OFET when a droplet of solution containing streptavidin molecules were used in it. The on-current was decreased further as the concentration of the streptavidin increased.

The estimated number of streptavidin and biotin molecules existed in the experiments resulted to the fact that the streptavidin molecules may have saturated the sensor during the experiment. However, the calculated diffusion time of streptavidin to travel from top level of o-ring to the surface of sensor refuted the statement by explaining that the experiment total time was not enough for all the molecules to reach the substrate thus a considerable number of them were not participating in the interactions. Furthermore, some of the streptavidin molecules might have been absorbed on the surfaces of o-ring and glass cover.

5.2. Future work

The results from this study and the previous one by Ismail Terkeşli [42], have proved the ability of planar OFETs to be integrated to microfluidic channels. As mentioned previously, the incorporation of microfluidic channels into these devices can bring innumerable advantages in terms of biological or chemical sensing. The microfluidic networks can ease the introduction or even immobilization of biomolecules into the sensor, and they can help to reduce the undesirable impacts of environmental factors like oxidation of organic materials in the atmosphere or prevent them from evaporating during long experiment times. Additionally, different biomolecules and concentrations of them can be pumped through these channels in a row to investigate the output characteristics of OFET in response to various biomolecules. Differential measurements can be achieved to remove common environmental effects. Nonetheless, there are some challenges and issues with the integration of microfluidic channels with the organic FETs, especially in the cases where the semiconductor layer needs to be patterned. These problems were also seen in the Ismail Terkeşli's study [42], where the OFET's performance was reduced after the patterning of P3HT film and fabrication of PDMS microchannel over the device. This

reduction in OFET performance can be a result of degradations in the polymer film since it is very sensitive when exposed to the solutions of fabrication processes.

To overcome this issue, we have studied a novel method to deposit organic polymer films on the device. This method can be used to form the polymer film after a microfluidic channel is fabricated. Electropolymerization or electrosynthesis of a polymer is a method in which a constant or varying voltage is applied to a conducting surface immersed in an electrolyte solution containing the polymer monomers and supporting solution for the process. This applied voltage can form polymer chains out of monomers and further deposition of these chains as a film of polymer can occur over the surface of the working electrode.

5.2.1 Electrochemical polymerization method

The details about the mechanism of the electrochemical polymerization are discussed in a thesis by Pinar Çamurlu [126]. The electrochemical polymerization mechanism is based on the oxidation of monomers. The oxidation requires one electron while excess of charge results in reversible oxidation of polymer or doping of polymer. Figure 5.1 depicts the steps happening in the process. In the very first step a radical cation is created due to the oxidation of a monomer. Because the electron transfer reaction tends to be faster than the diffusion of monomers from the bulk material, high concentrations of oxidized monomers will be present for further reactions.

Second step involves coupling of two radicals forming dihydro dimer cation. The following step lead to dimer with the loss of two protons and re-aromatization. The conjugated dimer generally has lower oxidation potential which means it can be easily oxidized forming a radical cation. This causes coupling with a monomeric radical or a radical cation with neutral monomer. Electrochemical and chemical processes are repeated until oligomers become insoluble in the electrolyte solution and precipitate into electrode surface. Monomer oxidation occurs continuously during the electro-polymerization due to the fact that conjugated monomers can be oxidized in less positive potential. The electro-polymerization is done typically by potentio-static (constant potential) or galvaostatic

(constant current) methods. Potentio-dynamic methods are used to obtain qualitative information about the reactions occurring in the first stage of polymerization process and study the behavior of the polymer film after electro-deposition. The electrochemical methods affect the morphology, appearance and adhesion of the polymer.

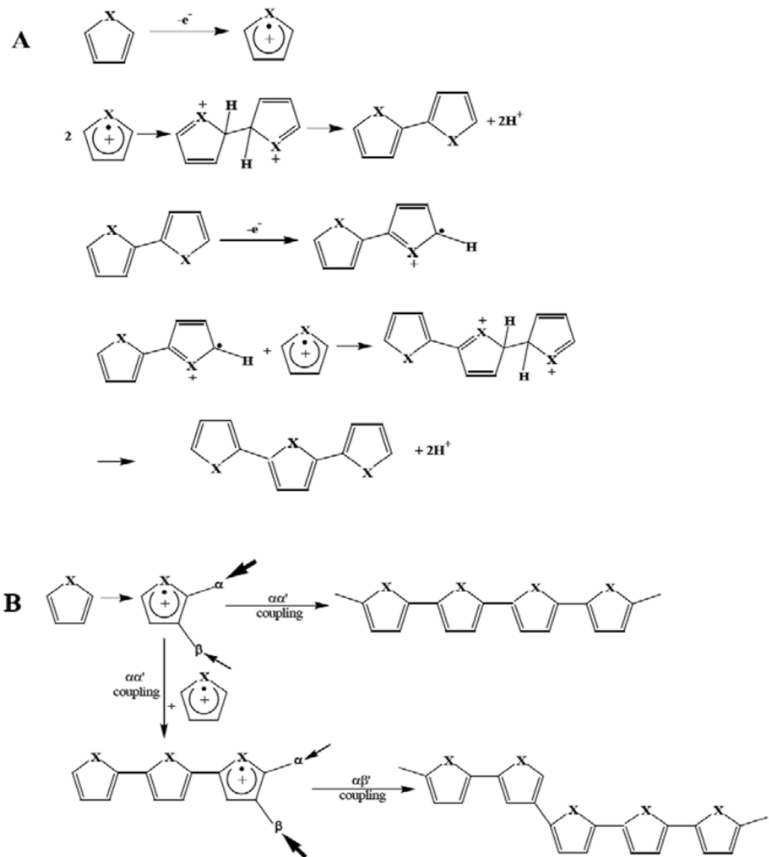


Figure 5.1. Illustration of the steps in the electropolymerization [126].

Figure 5.1 also shows two possible linkages between polymer monomers: The $\alpha - \alpha$ linkage and $\alpha - \beta$ linkage. The activity of α or β position in the monomer decides the linkage, which will occur during the polymerization process. Some polymers naturally have more active α positions, which will lead to a $\alpha - \alpha$ linkage. This kind of linkage proposes formation of a high quality and more uniform polymer chains. However, the $\alpha - \beta$ coupling cannot be avoided totally and occurs in the cases that $\alpha - \alpha$ coupling is not achievable due to some defects in the monomers or the supporting solution. This coupling can also result in formation of fine polymer chains.

Recently, the so-called electrochemical synthesis process have been used to from different kinds of polymer films. Among these polymers, polythiophene and its derivatives have been prepared and synthesized electrochemically with various methods in a significant number of studies [127-129]. Their remarkable electronic, magnetic and optical properties which have found them a wide range of applications in electronic and non-electronic fields as well as their solubility in the organic solvents have made them one of the most popular polymers used in electropolymerization processes. Electropolymerization of polythiophene from two different monomers over a gold substrate using cyclic voltammetry in a three electrode configuration was reported [130]. In a similar work, electrodeposition of polythiophene films on the selected areas over a gold surface was investigated [131]. Cyclic voltammetry (CV), a method in which a reference electrode made from platinum, a counter electrode normally made from Ag and a working conductive electrode – which in the majority of experiments is made from gold- are used to apply the necessary voltages, was exploited in these studies to achieve a fine quality film and monitor the deposition of it by analyzing the oxidation points. This advantage of CV method was utilized in a study to characterize obtained polythiophene films from electrochemical polymerization process [132].

Electropolymerization method is also exploited vastly in the field of biosensors as well as other fields. It is considered to enhance the polymerization of electronically conductive polymers in the biosensor devices. A tutorial review by Serge Cosnier and Michael Holzinger [133] talks about direct electropolymerization of biomolecules as an alternative method for immobilization and entrapment of them. This method is stated to be simpler and more convenient methodology and also immobilize more number of biomolecules over the sensor surface. The older reports and studies which have used this method for the biomolecules entrapment are given in the mentioned review. Another review by Serge Cosnier *et al.* [134] opens up novel perspectives about the concept of biomolecules immobilization through the electrochemical polymerization method. Three distinct approaches for the entrapment of molecules into the electrochemically generated polymer films are described in this review. As instances of practical conducted experiments, Gerard Bidan *et al.* [135] has investigated the electrochemical copolymerization of pyrrole and oligonucleotides bearing on their 5' end a pyrrole moiety to prepare DNA matrices. Furthermore, a more interesting work by Serge Cosnier *et al.*

[136] has reported electrochemical synthesis of biotinylated pyrrole film over a conducting electrode.

Considering the fact that the integration of microfluidic channels have degradation effects over the deposited polymer film performance, we decided to take advantage of using electrochemical synthesis method to allow formation of polymer films after fabrication of microfluidic channels. The deposition solution can be pumped into the microfluidic channel and by applying the correct form and amount of voltage to the working electrodes the polymerization can be achieved.

In the first step to start experimenting the electropolymerization, we used pure lithium perchlorate ($LiClO_4$) as the supporting agent which was used to prepare the electrolyte solution. Acetonitrile was selected as the solvent and $LiClO_4$ was added to it with a molarity of $0.1M$. The deposition solution was $0.2M$ of 3-hexylthiophene monomer dissolved in the $0.1M LiClO_4/ACN$.

The approach that we adopted for this experiment consisted of applying a varying voltage from zero to 1.8 V (since the oxidation potential of polythiophene was 2 V, we wanted to avoid any formation of oxidized layers by choosing lower voltages) to the gold drain and source electrodes and putting a droplet of deposition solution inside an o-ring. The counter electrode which was made from tungsten was inserted into the solution and a small plastic stretching film was utilized as the cover to avoid evaporation of solution during the experiment.

After conducting the experiment for couple times to observe the formation of polythiophene film to understand a relation between deposition rate and the voltage and the time, we were able to deposit a thin layer of conducting polythiophene polymer on and in the surroundings of the working gold electrodes. Figure 5.2 shows the formed polymer layer whose color is in red. The deposition has showed a higher rate mostly in the places adjacent to the counter electrode, since there would be higher electric fields. There are some uncoated parts in the experiment because of some resistive voltage drop in the solution. Figure 5.3 gives a closer look at the covered electrodes.

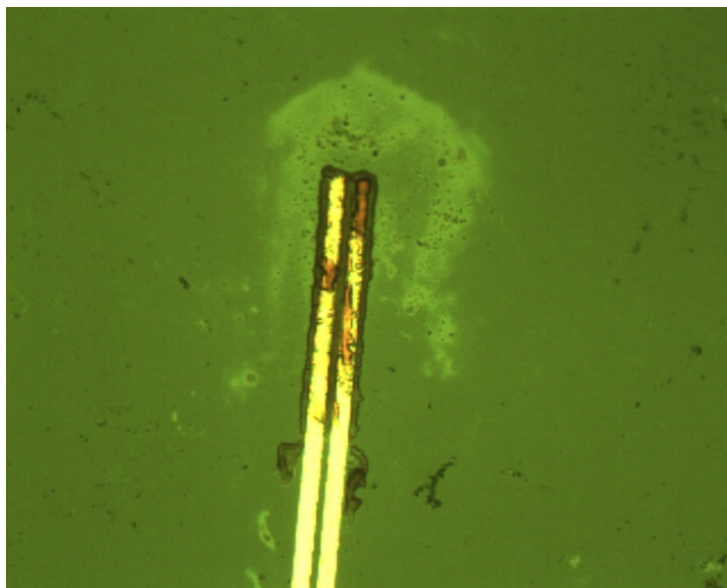


Figure 5.2. The drain and source electrodes after electropolymerization experiment.

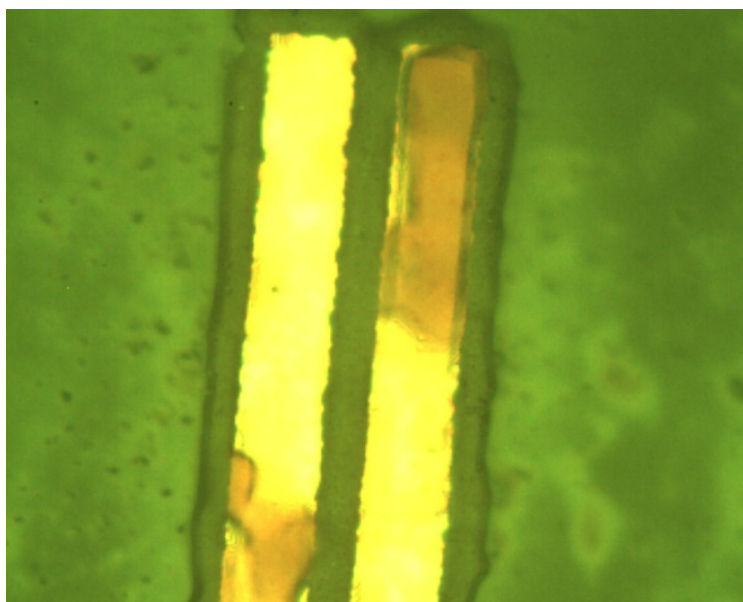


Figure 5.3. Closer look at the same electrodes in Figure 5.2.

The results from this experiment prove the great potential of the electrochemical polymerization to be exploited in the future projects. After fabricating the microfluidic channels over the OFET, the deposition solution can be pumped into the channel and varying potential can be applied to the source and drain electrodes to selectively deposit polythiophene or even biotinylated polythiophene over and between the electrodes. The

results also show that the gap between the two working electrodes can be filled with deposited polymer.

Consequently, electropolymerization methodology can provide an effective solution to the issues related to the incorporation of microfluidic channels with the organic FETs. This technique can be investigated in future works to introduced microfluidic networks into the studied sensor cell and build a much more reliable and sensitive biosensor device based on the WGOFET.

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