Liquid-gated field effect transistors based on single-walled carbon nanotubes : investigations of nanotubes-biomolecular interactions and ultra-sensitive biosensors

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Liquid-Gated Field Effect Transistors based on Single-Walled Carbon Nanotubes: Investigations of Nanotubes-Biomolecular Interactions and Ultra-Sensitive Biosensors

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A Thesis submitted to the Nanyang Technological University in fulfillment
of the requirement for the degree of Doctor of Philosophy

by

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School of Materials Science and Engineering

2009

*Research is to see what everybody has seen, and to think
what nobody else has thought*

Albert Szent-Gyorgyi (1937 Nobel Laureate in Medicine, 1893 – 1986)

Abstract

Detection and analysis of biomolecules is of paramount importance in the areas of medical diagnostics, environmental monitoring and defense which often requires rapid, low-cost, portable, and high-sensitivity analysis. In this thesis, an alternative approach to attain these attributes is demonstrated by employing single-walled carbon nanotubes (SWCNTs) based liquid-gated field-effect transistor (LGFET) with an integrated microfluidic channel.

To address the need for a simple and robust fabrication step, a simple lamination process is described in this thesis for the production of the device which requires merely two materials: SWCNTs and poly (dimethyl siloxane) (PDMS), an elastomer. This uncomplicated production process, which involves soft lithography, alleviates the need for photolithography altogether, besides the initial requirement of mold preparation. The integrated microfluidic channel offers advantages, such as: controlled delivery of the liquid sample, minute requirement of sample volume, and ability to perform kinetic measurements amongst others. The biomolecular interaction is then studied in real-time and label-free fashion within the aqueous liquid environment by monitoring the conductance of the semiconducting carbon nanotubes network.

The size similarity of the nanotubes with most biomolecules and electronic properties of SWCNT derived from its hollow geometry renders the SWCNTs a potential candidate for high-sensitivity biosensing. To gain understanding about the interaction between the biomolecules and the nanotubes, a series of experiments have been performed to study the interaction between Bovine Serum Albumin (BSA) protein and the nanotubes, with a focus given to the reciprocal effect of such interactions: protein conformation change and conductance of the LGFETs. The carboxylated nanotubes inflicted a large conformational change in the protein than its pristine counterpart, thus, resulting in differential signal level in the transistor based kinetic measurement.

This observation underscores the importance of the precise surface chemistry of the nanotubes in developing a biosensor.

The application of SWCNTs LGFET in biosensing was investigated in the detection of 2, 4-dichlorophenoxy acetic acid (2, 4-D) herbicide in both buffer solution and soil extract by employing competitive immunoassay. The study suggests that the size of the bioanalytes determines the type of protocol that can be employed for the kinetic detection. Additionally, the experiment also highlights that lower ionic-strength solution, such as soil extracts, helps in signal amplification thus facilitating detection limits down to femto-molar concentrations. A similar ionic-strength dependency of the signal level is also found in the successive experiments of adsorption of poly (L-Lysine) (PLL) in different ionic-strength solutions.

Additionally, in order to improve the device sensitivity, a pulsed-gating technique has been devised to extend device operation to voltage ranges beyond the electrolysis boundary. By kinetically preempting the electrolysis reaction, the extended operating window allows to probe effects of capacitance as a sensing mechanism at high gate bias values, with simultaneous signal amplification and corollary improvement in the sensitivity of the device to detect biomolecules down to atto-molar concentration levels.

Acknowledgements

"The important thing is not to stop questioning. Curiosity has its own reason for existing."

Albert Einstein (1921 Nobel Laureate in Physics, 1879 – 1955)

So it is said that even a journey of a thousand miles starts with a step, and so does this post-graduate project, which have become a personal journey for me to grow scientifically, and psychologically; and which have been both exhilarating and stressing at times. Looking back, however, I could not imagine reaching this far without the support of numerous people that have given to me directly and indirectly; and the lessons they give to me along the way through various opportunities.

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Singapore, March 30, 2010

I Putu Mahendra Wijaya

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Chapter 1 Introduction

Nanotechnology as a nascent field has sparked the interests of research academia and practitioners around the world from diverse backgrounds. Part of the reason behind this heightened interest is a realization that nano-materials may hold the key to advance the technology useful for humanity in the future. This promise of nanotechnology stems out directly from the unique properties of nano-materials. Uniquely positioned at the juncture between classical and quantum physics realm, nano-meter scale invoke many interesting behavior of otherwise common materials ^[1]. Plainly, all these novel properties open up a plethora of interesting opportunities. One of these is the possibility to integrate biological world with the world of electronics, in general, and design of novel sensing strategies in biosensors, in particular.

Current molecular sensing, such as Enzyme-Linked Immunosorbent Assay (ELISA) ^[2], Surface Plasmon Resonance (SPR) ^[3], and electrochemical methods ^[4, 5] are sensitive enough to probe many of the necessary biological reactions. However, these techniques generally rely on complex protocol ^[6] which arises because the size of most biological molecules is much smaller than the wavelength of light, and hence additional steps must be taken if the reactions were to be studied with optical mean. On another hand, the complication of electrochemical methods arises because not all biomolecules are electro-active and hence necessitates electrochemical molecular tagging to study such biological reactions electrochemically ^[4, 5].

Fortunately, many interactions that take place between the biomolecules are electrical in nature. Hence, this information opens up the possibility to study the biological interaction with electronic measurements, which do not require labeling and simplify the measurement protocol and allows to probe in real-time of such interactions.

Single-walled carbon nanotubes (SWCNTs) transistor in this case offers an interesting route to sense such biomolecular event electronically. As I will discuss in more detail shortly, the single atomic layer typical of all SWCNTs makes the nanotube extremely sensitive toward electrical disturbance that originates from the biomolecular interaction. In the transistor format, this potential disturbance influences the conductivity of the nanotube and hence its current level, as reported in the literatures ^[7-15].

Briefly, the concept of nanotube transistor was first coined in 1997 ^[16], a shy six years away after the SWCNT discovery. The realization of utilizing the nanotube as sensing element, however, only comes in 2001 when Kong, Chapeline, and Dai ^[17] reported for the first time the use of SWCNT as a molecular hydrogen sensor. A year afterward, Shim et al ^[18] then reported a functionalization method which covalently links the nanotube with biomolecules, a necessary step to introduce selectivity in nanotube biosensing. Thereafter, the progress has been quite rapid. In 2003, Besteman et al ^[19] demonstrated that when a nanotube is coated with glucose oxidase, an enzyme; the resulting transistor is capable to sense oxidation of glucose and pH changes real-time in liquid environment. In the same year, Star et al ^[11] independently reported the use of SWCNT transistor to detect biotin-streptavidin binding in dry-state measurement. A year afterward, Star et al ^[20] also reported that the nanotube transistor is also capable to sense the degradation of

starch into glucose with amiloglucosidase enzyme also in dry-state measurement. In 2006, Star et al ^[21] and Tang et al ^[12] showed a label-free detection of deoxyribonucleic acid (DNA) hybridization with the SWCNT transistor.

Despite of all of these advances, however, the origin of sensing mechanism has become a challenging problem. For example, in their paper on DNA hybridization, Star et al ^[21] have argued that the transistor signal originates as a result of charge transfer between the biomolecule and the nanotube. This is in contrast with the mechanism which is proposed by Tang et al ^[12] who argue that the signal comes predominantly from the biomolecule interaction with the electrodes instead. In the ensuing years, several works have been done as attempts to answer this quandary. For example, Gui et al ^[9] have tried to investigate this problem with the aid of DNA intercalator, and propose that the signal is originated primarily from the contact area between the electrode and the nanotube. In 2006, Larimore et al ^[22] also reported that the choice of gate electrode materials could influence the result from the transistor biosensing, and hence may need to be taken into account when analyzing and comparing data from previous reports. Furthermore, she shows that employment of standard reference electrode as the gate electrode could actually eliminate this unnecessary and complicating variation. In year 2008, Heller et al ^[23] showed that there are four possible mechanisms that can contribute to the SWCNT transistor biosensing, namely: (1) electrostatic gating, (2) Schottky barrier modulation, (3) capacitance changes, and (4) mobility modulation. Each of these mechanisms will be discussed in greater detail in the subsequent chapters.

1.1 Research objectives

The absence of agreement on the sensing mechanism of the nanotube transistor biosensor, as highlighted in the review above, clearly hampers the potential of the development of the nanotube transistor for biosensing applications. Hence, the prime objective of this project is to answer this challenge which encompasses aspects, such as:

1. Fabrication and characterization of a SWCNTs based liquid-gated field-effect transistor (LGFET)

The fabrication of the LGFET, as well as the system preparation plainly becomes an integral part in the investigation of the sensing mechanism behind the biosensing. In particular, it is important to devise a simplistic and robust method allowing to produce the device in sufficient number for the subsequent experimentations. Hence, a simple method to fabricate such a device shall be described in this thesis, which involves a soft lithography process, and hence eliminates the need for a photolithography steps altogether, beyond the initial preparation of hard mold. Equally important is the checking steps to make sure that the devices obtained are indeed transistors, which can be easily tested by performing basic transistor characterization measurements, such as: output and transfer characteristic measurements as detailed further in the thesis.

2. Experimental and simulation studies of the interaction between the biomolecules and the nanotubes

The interaction between the nanotubes and the biomolecules forms the basis behind the sensing ability of the LGFET. Hence, in order to appreciate the sensing mechanism of the

transistor device, it is important to understand the nature of such interaction, and hence its influence on the signal level in the LGFET. A series of experiments shall be described later in the thesis that aims to improve our understanding of the nature of such biomolecule/nanotube interaction which employs spectroscopic, electrical, and simulation studies and its resulting reciprocal effects to both the biomolecule and the nanotube.

3. Demonstrate the applicability of the LGFET as a biosensor and fundamental studies to highlight their sensing mechanisms

Having gained understanding of the interaction between the nanotube and the biomolecules, the potential applicability of the LGFET for biosensing purposes naturally forms an aspect of the objectives to investigate the sensing mechanism. In specific, the ability of the LGFET to identify important bioanalytes in both buffer solution and real-life matrix shall be studied in the thesis. Factors that influence the sensitivity of the device shall be investigated, as well as the fundamental studies needed to elucidate its sensing mechanism(s).

4. Investigate suitable amplification strategies to yield a highly sensitive biosensor

As in any other sensing technologies, the figure of merit which determines the quality of signal coming from the LGFET typically is described by its signal-to-noise ratio (SNR) that can be improved by either reducing the noise level and/or amplify the signal level. Hence, in order to press forward the sensitivity of the LGFET further, a technique shall be introduced in the thesis that allows us to extend the operating window beyond

electrolysis limit of ± 1 volt, and hence attaining higher current level with the concurrent signal amplification and the consequent improvement in the sensitivity of the device. As shall be described in detail in the thesis, the attainment of higher current level also helps to illuminate some of the sensing mechanism which is observable only at higher gate bias window.

1.2 Thesis Outline

The basic physics that explains the working principle behind conduction of electrical current in the nanotubes is discussed in Chapter 2. This chapter covers topics such as: energy-momentum diagram of graphene and how it can be extended to explain the electrical behavior of nanotubes, reasons behind duality in electrical behavior: semi-conducting and metallic; that is exhibited by the SWCNTs, and the different charge transport mechanism that can take place in the nanotubes. In addition, the different techniques that can be employed to grow SWCNTs shall also be discussed in Chapter 2. The catalytic chemical vapor deposition (CCVD) technique which uses ethanol as the carbon precursor to grow nanotube shall be described in more detail in this chapter. The in-house system that is engaged in the CCVD method, as well as its working flow and possible molecular process that takes place inside the system shall also be discussed in this segment.

Subsequently, the operating principle behind the nanotube transistor shall be discussed in more detail in Chapter 3. As a start, the link between physical concepts that explain the conducting behavior of nanotubes and the working principle of SWCNT transistor of

both types: liquid-gated and substrate-gated; shall be established in this chapter. Subsequently, the process flow that is engaged in the making of the nanotube transistor and the program design of the measurement system that accompanies it shall be discussed. Additionally, the chapter also covers the simulation results that explore the various possibilities on how a biomolecule interact with nanotubes, and the effect of these various interactions on the I_{DS} - V_G of the transistor. Finally, results from basic characterization of a transistor: I_{DS} - V_{DS} , I_{DS} - V_G , and I_{DS} vs. leakage current – all are sine qua non to verify the transistor device; shall also be examined in this chapter.

In Chapter 4, the reciprocal effect of protein interaction with carbon nanotubes shall be examined in more detail. The model protein, bovine serum albumin (BSA) here is examined upon its interaction with pristine and carboxylated nanotube and its consequence toward both the conformation of the protein, and the conductivity of the nanotube shall be studied. Furthermore, this chapter shall also discuss how local environment that surrounds the pristine and carboxylated nanotubes influence the conductance greatly. The results obtained from this experiment indicate the possibility of using nanotube transistor as a device to investigate the conformational changes a protein can adopt when in contact with the nanotubes, in tandem with other characterization techniques, such as: fluorescence spectroscopy, and circular dichroism; and simulation studies.

Besides the effect of biomolecular interaction with the nanotubes, the response of LGFET biosensor is also influenced by the aqueous environment in which it usually operates. Hence, the effect of ions in the liquid media shall be discussed in greater depth in Chapter

5. Biomolecules dissolved in liquid media have either charge or certain degree of polarity to render them being hydrophilic. The interference of this innate electric field with the charge transport in the nanotube becomes the predominant origin of the biosensing signal in the nanotube transistor. Unfortunately, this same charge and polarity that resides on the surface of the biomolecules could also attract counter-ions that are dissolved in the aqueous medium. Hence, the effect of these counter-ions on to the nanotube signal shall be discussed in great depth in Chapter 5.

The demonstration of nanotube transistor as a biosensor then is established in Chapter 6, in which the transistor here is engaged in detection of herbicide 2, 4-dichloro phenoxy acetic acid (2, 4-D). Here, it shall be revealed that the details on sensing strategy that can be applied are dictated by several factors, such as: the ionic-strength of the solution, and the size of the analytes with respect to the size of the probe molecules. In the case of 2, 4-D, the large disparity between the size of the analytes and the antibodies necessitates the engagement of competitive immuno assay as the most suitable protocol to detect the antigen. Furthermore, this chapter also discuss on how the reduction of ionic-strength in soil extract could actually helps the nanotube transistor to attain a very low detection limit, up to Femto-molar concentrations, of the 2, 4-D.

The operation of nanotube transistor in liquid media offers an advantage in biosensing applications in that it allows the device to probe the biomolecular interaction in their most native environment. However, the presence of water molecules also put a natural barrier on the possible V_G bias that can be applied in traditional measurement setting. That is, the V_G applied can not exceed $|V_G| > 1$ volt window in normal operating DC sweep, to avoid

the electrolysis of water. Hence, in Chapter 7 one possible method shall be discussed: pulsed-gating; to extend this operating window, and at the same time allows for signal amplification.

Finally, Chapter 8 summarizes the results that have been discussed in the previous chapters, as well as offers an insight on the possible future works that can be extended from this present project. In particular, the chapter offers various possible methods that may be applied in the future as alternatives for signal amplification. This amplification strategy, decidedly will play an important role in the future to push the sensitivity and detection limit of the nanotube transistor even to a lower concentrations, which may be critical for many applications. Additionally, the chapter also offers some alternatives that can be attempted to further improve the functionality of the LGFET even further, with examples such as: integration of the LGFET with other microfluidic technologies, substitution of the SWCNT with other types of semiconducting materials, such as: graphene, organic semiconductors, etc; and electronic circuit application of the LGFET.

Chapter 2 Single-Walled Carbon Nanotubes

In nature, SWCNTs are typically produced as a by product of a combustion reaction. Hence, human may have produced SWCNT ever since we are able to create fire and burn biomasses to derive our energy. However, Iijima ^[1] in 1991 reported SWCNT scientifically for the first time through his transmission electron micrographs and analysis. Conceptually, at least, a SWCNT can be derived from rolling a graphene sheet, shown in Figure 2.1(a), into a cylinder. Plainly, the result is a nanometer-scaled tube with a length typically in the order of micrometer, although a SWCNT with length up to 4 cm has been reported ^[24].

SWCNT has many interesting properties owing to a combination of its geometry, and to the fact that it is made solely from carbon. These interesting properties have made SWCNT one of the hottest topics in physics since 2006 ^[25]. For example, SWCNT has been interesting theoretical subject because of its quasi one-dimension geometry ^[26]. It also offers interesting application in the field of photonic ^[27], and nano-mechanics ^[28]. The small size and unique electronic properties also make SWCNTs very good candidate for chemical sensing, as demonstrated by Kong et al ^[17].

Furthermore, the hollow geometry and all-covalent bond also give SWCNTs extraordinary mechanical property, coupled with extreme low density. Wong ^[29] reported that the tensile modulus of the SWCNT can be as high as 1 TPa and Lu ^[30] showed its tensile strength is as high as 20 GPa. Such interesting mechanical properties alone have resulted in numerous SWCNT studies ^[31-34]. Safadi ^[34], for example, found that

incorporating a small weight percentage of multi-walled carbon nanotubes (MWCNTs) approximately 2.5 wt% into polystyrene matrix could improve its tensile modulus by 100%.

Figure 2.1 (a) Graphene sheet and (b) SWCNT. Graphene sheet is made entirely from carbon in honeycomb arrangement. Rolling a graphene sheet into a tube forms a SWCNT^[15].

Influenced by the geometry and composition of SWCNTs, their electrical properties are also outstanding. A SWCNT can show either metallic or semi-conducting behavior depending on the way the graphene sheet is rolled. Before one can understand how this is possible, one need to study a little further about graphene energy-momentum diagram and rolling indices in SWCNT, and how the two parameters interact to give rise to the observed interesting electrical property.

2.1 Energy-momentum diagram of graphene

Figure 2.1(a) shows that a graphene sheet has many carbon atoms in honeycomb

arrangement. The periodicity of the structure exerts some modification on the movement of electrons, as compared when they travel freely in vacuum. This fact leads to a modified version of Schrodinger equation, which takes into account of such a periodicity. The solution of such modified Schrodinger equation in crystal solids, in general, is known as Bloch wave function.

Equation 2.1

$$\psi_{n\vec{k}}(\vec{r}) = e^{i\vec{k} \cdot \vec{r}} u_{n\vec{k}}(\vec{r})$$

As observed from Equation 2.1, the Bloch wave function $\psi_{n\vec{k}}$ is a product of the wave function of the electron $e^{i\vec{k} \cdot \vec{r}}$ and a periodic potential from the crystal $u_{n\vec{k}}(\vec{r})$. The modified Schrodinger equation which produced equation (2.1) looks like as below^[35].

Equation 2.2

$$[H(\vec{k})]\psi_0 = E\psi_0$$

In Equation 2.2, the Eigen values of a quantum system are influenced by a Hamiltonian that operates on the system. Hamiltonian is defined as a summation of all types of energy of a particular wave function. Hence, the Hamiltonian that operates in a graphene should reflect some periodicity owing to its potential energy component. Indeed, such a feature is found in the following Hamiltonian defined for crystalline solids.

Equation 2.3

$$\left[H(\vec{k}) \right] = \sum_m H_{nm} e^{i(\vec{k} \cdot \vec{a}) \delta_{mn}}$$

Equation 2.3 basically tells that the Hamiltonian of crystalline solids, such as graphene, is a result from summation from all of the atoms situated in its unit cell. The term δ_{mn} found in Equation 2.3 is to denote the delta function which takes a value of one when $m=n$ and zero otherwise. The delta function in this equation represents the discrete nature of atoms.

The wave function for graphene, as shown in Equation 2.1, comes from solving Equation 2.2 and Equation 2.3. The Eigen values, or energy level, for graphene then can be solved simultaneously, as shown below.

Equation 2.4

$$E = E_{2p} + \left| H_0(\vec{k}) \right|$$

The component E_{2p} in Equation 2.4 represents the p_z -orbital energy of graphene, and the component H_0 represents dispersion relation in graphene.

Equation 2.5

$$\left| H_0(\vec{k}) \right| = \gamma_{\pi\pi} \sqrt{1 + 4 \cos\left(k_x \frac{a\sqrt{3}}{2}\right) \cos\left(k_y \frac{a}{2}\right) + \cos^2\left(k_y \frac{a}{2}\right)}$$

The term $\gamma_{\pi\pi}$ in Equation 2.5 represents the carbon-carbon interaction strength in graphene. And finally, the term k_x and k_y represent the wave vector in x- and y-direction,

respectively.

Equation 2.5 is usually called dispersion relation in crystalline solids. However, recalling that momentum in quantum mechanics is defined as

Equation 2.6

$$p = \hbar k$$

, Equation 2.5 sometimes is also known as the energy-momentum (E-k) relation in crystalline solids. The E-k diagram of graphene then can be obtained through plotting Equation 2.5 within the first Brillouin zone, as shown in Figure 2.2 below.

Figure 2.2 (a) E-k diagram of graphene. Energy-momentum diagram of graphene can be obtained by solving Schrodinger equation with Hamiltonian suited for graphene. Depicted here are two solutions from the equation. The lower part (blue) represents the valence band and the top part (red) represents the conduction band. (b) Contour of E-k diagram.

There are several important symmetrical points in the E-k diagram worth noting, shown

in Figure 2.2(b). First is the Γ point which lies at the zenith point in the conduction band. Second, there is the M point, where a local minimum is found, and finally, the K and K' points, where the conduction and valence band are met.

The presence of K and K' makes graphene a zero band gap material. Furthermore, these points also have consequence toward the electrical behavior of SWCNTs. To understand this fact, one first need to understand the concept of rolling indices and zone-folding condition, as explained in the following section.

2.2 Rolling indices: rolling a graphene sheet into a SWCNT

Rolling a graphene sheet into a SWCNT is like rolling a piece of paper into a tube: you can do it with many ways. However, the way a graphene sheet is rolled into SWCNT can have a crucial impact on its electrical behavior. Hence, some kind of naming system that states the rolling direction is needed.

Indeed, previous works on SWCNTs have created such a naming system ^[36, 37]. To understand this nomenclature system, first one needs to define an origin at one of the carbon atom, as shown in Figure 2.3. From there, two basis vectors $\mathbf{a}_1$ and $\mathbf{a}_2$ that span the two “zigzag” directions are then created. Hence, any rolling direction in the graphene sheet can be stated in term of these two vectors.

Equation 2.7

$$\vec{r} = m\vec{a}_1 + n\vec{a}_2$$

Alternatively, the rolling direction can also be stated in the form of indices (m, n) which is unique for a particular SWCNT family. Hence, with this system, the SWCNTs can be grouped into three broad categories (Figure 2.3): zigzag, arm-chair, and chiral. First, note that all SWCNTs which have rolling indices $(m, 0)$ or $(0, m)$ always have zigzag carbon pattern in the circumference direction. Second, note as well that all SWCNTs which have rolling indices (m, m) or (n, n) always have armchair carbon pattern in the circumference direction. Only SWCNTs with arbitrary rolling indices (m, n) does not have this regularity. Hence, broadly speaking, SWCNTs can be grouped into of armchair, zigzag, or chiral type based on this special regularity in the indices.

Figure 2.3 (a) honeycomb structure of graphene sheet (b) (8,8) armchair, (c) (8,0) zigzag, and (d) (10,-2) chiral SWCNT^[38].

2.3 Quantum confinement and zone-folding

Nanometer is a unique length scale because it is the dimension where the laws of quantum mechanics meet the law of classical physics. Hence, a rich physical phenomenon could happen owing to this interfacing. Indeed, the similarity between the dimension of a system and an object, in it self, could lead to interesting applications, exploiting the innate interplay which takes place between the two. For example, lights have been manipulated in a chaotic quantum confinement of a spiral *micro*-cavity for development of micro-lasers ^[39]. At the nano-scale system, such as SWCNT, similar quantum confinement could also take place to the electron wave function.

Quantum confinement comes about because of a restriction imposed on the movement of the electrons due to physical boundary in the nano-scale systems. Such restrictions manifests itself in the Schrodinger equation and results in observed discrete energy level. In the case of SWCNT, the quantum confinement is produced due to restriction on the electron movement on the rolling direction.

The electron wave length in the rolling direction is restricted to those values whose integer multiples of its half wave length is equal to the circumference of the SWCNT. Or, mathematically, this statement can be written to:

Equation 2.8

$$n \frac{\lambda}{2} = \pi D$$

Since wave vector is defined as $k = \frac{2\pi}{\lambda}$, Equation 2.8 can be recasted into

Equation 2.9

$$k_{circ} = \frac{n}{D}$$

(a)

(b)

Figure 2.4 (a) zone-folding condition results in not all energy in the E-k diagram are allowed. (b) zone-folding condition observed from cross-sectional view^[40].

As a result of quantized k_{circ} , not all energy in the E-k diagram is allowed in SWCNT – the so-called zone folding condition. In the E-k diagram, this quantization appears as slicing planes along the rolling directions, shown in Figure 2.4(a). If this sliced E-k

diagram is viewed from the cross-section, shown in Figure 2.4(b), it reveals the E-k diagram for a SWCNT of this particular rolling direction.

Additionally, note that one of the slicing planes passes through the K and K' points in Figure 2.4. This intersection results in metallic SWCNT. However, at some other cases, the slicing plane may not pass through neither K nor K' point at all. In such a case, the SWCNT would show a semi-conducting behavior. Hence, the rolling direction of a SWCNT determines whether it behaves like metal or semi-conductor. It has been shown previously^[41] that 2/3 of SWCNT are semi-conductors, while 1/3 of them are metallic. Furthermore, Reich et al^[42] have also shown that all arm chair SWCNT are metallic in nature.

In this thesis, I am interested on how semi-conducting SWCNT conduct electrons in the presence of metal/semiconductor junctions. This knowledge is important for the understanding of the behavior SWCNT LGFET in biosensing applications. The next section will briefly discuss about how electrons are transported in a semi-conducting SWCNT.

2.4 Electronic transport in semi-conducting SWCNT

Early theoretical works^[43-45] showed that SWCNTs can conduct charge carrier ballistically. Tans et al^[16] proved this experimentally and showed that coherent electron transport through SWCNT can be retained up to a distance at least 140 nm, or even up to 10 μ m in arm-chair SWCNT^[46], provided that the tube is minimally disordered. Additionally, Li et al^[47] showed that ballistic transport is also possible in

multi-walled carbon nanotube (MWCNT)

However, factors such as: imperfect structure of SWCNT, localization effect ^[46], and Schottky barrier ^[48]; makes realization of this ballistic transport in actual SWCNT experiment difficult.

Hence, in practice, it can be assumed that the SWCNT tube follows the classical Drude's model^[49] of diffusive transport which states that the current density in conductors is nothing but a flux of charged particles.

Equation 2.10

$$J = env$$

In linear regime, the velocity in Equation 2.10 is proportional to the applied electric field $v = \mu |\vec{E}|$, with mobility (μ) as the proportionality constant. Hence, Equation 2.10 can be recast into

Equation 2.11

$$J = \sigma |\vec{E}|$$

Where σ represents the conductivity, which is defined mathematically as

Equation 2.12

$$\sigma = en\mu$$

I will reveal later that a modified version of Equation 2.12 can also be applied to the

effect of diffusive transport in SWCNT LGFET^[23].

In addition to the bulk property, the contact between the SWCNT and the metal electrodes also play an important role in electronic transport behavior of the SWCNT. Previous theoretical works on SWCNT transistors^[23, 50] have pointed out that the effect of contact in single SWCNT transistor behavior is more appropriately addressed with the so-called Landauer formula.

Equation 2.13

$$I_{ds} = \frac{4e}{h} \int_{-\infty}^{\infty} T(E) (f_S(E) - f_D(E - eV_{ds})) dE$$

In the presence of both Schottky barrier and diffusive transport, the resultant conductance from the mechanism can be depicted electrically as two resistors^[23], one from each mechanism, connected in series. Hence, when these two mechanisms are in operation, the resultant conductance of a SWCNT FET can be calculated with

Equation 2.14

$$\frac{1}{G_R} = \frac{1}{G_{Schottky}} + \frac{1}{G_{diffusive}}$$

Equation 2.14 shows basically that the G_R would be predominated by the weaker conductance between $G_{Schottky}$ and $G_{diffusive}$. The implication of this behavior in the experiment will become very clear in Chapter 3 when I discuss a simulation on liquid-gated field effect transistor. After gaining an understanding about the basic physics

behind the SWCNTs, I shall now turn the topic to methods to grow SWCNTs and manipulate them.

2.5 Growth of SWCNT

In 1985, during their course to study the chemistry of stars, a series of experiment were done in University of Sussex which leads to a serendipitous discovery of buckminsterfullerene ^[51], or C₆₀, which has a geometry of a soccer ball. The inventors: Kroto, Heath, O'Brien, Curl, and Smalley; had devised a set up, which later to be known as 'electric-arc discharge' to simulate the condition of stars. Later in 1991, Iijima ^[1] devised the same set up and reported that C₆₀ actually has a tubular cousin. In his paper, Iijima called the tubular cousin of C₆₀ as 'helical microtubules of graphitic carbon', which is now more famously known as the SWCNT.

Hence, historically, the first method reported to grow SWCNT was the electric-arc discharge. In this method, two graphite electrodes, anode and cathode, were separated at a distance approximately 8 mm in Helium environment. A high DC voltage then is applied across these two electrodes which induces break down of the Helium atoms, and create plasma.

The high energy density of the plasma leads to sublimation of the anode electrode. Over the course of time, the sublimated carbon atoms then undergo a de-sublimation stage at the cathode. This de-sublimation results in polymorphs of carbon, such as: amorphous carbon, graphite, SWCNT, MWCNT, and carbon nanohorn.

Of course, the details of the parameters in the arc-discharge affect the type, quality and the property of the resulting SWCNT. Knapp et al ^[52], for example, showed that application of a certain level of current is necessary to initiate the growth of the SWCNT. However, they pointed out that if the current level is too high, it may damage the SWCNT instead. Wang et al ^[53], on another hand, tried to replace the Helium environment with NaCl aqueous solution. They showed that if NaCl is used during the arc-discharge process, only SWCNT is formed during the process. In addition, Yumura ^[54] reported the effect of Helium pressure in the arc-discharge process. He pointed out that if the Helium greater than 500 psi is applied; only MWCNT is obtained. On another hand, if the pressure is reduced to less than 100 psi, a large quantity of C₆₀ is obtained.

Alternatively, SWCNTs can also be grown by a method called laser ablation. In this method, a graphite rod, alloyed with some catalyst metal, such as: Cobalt, Iron, or Nickel; is inserted in a quartz tube, which is connected to a flowing gas. A beam of high energy laser then is shined on to the rod. The laser vaporizes the surface of the graphite and frees the catalyst metal particles. Visually, the high energy vapor is observed as a plume in the quartz tube. The growth process then takes place within the gas environment. The resulting SWCNTs then can be collected at the outlet of the quartz tube ^[55]. In a twist to laser ablation method, Vander Wal et al^[56] uses the laser to evaporate the catalyst metal instead. They then apply flame enriched with carbon species to induce the growth of the SWCNT. Hence, Vander Wal et al have shown that SWCNTs can be grown even at a complex chemical environment, such as flame.

The arc-discharge and laser-ablation methods to grow SWCNTs involve high temperature

because of the need to sublime and de-sublime a graphite rod. Alternatively, the SWCNTs can also be grown with catalytic chemical vapor deposition (CCVD). In brief, the CCVD method grows the nanotubes with the aid of catalysts, and hence lowering down the formation energy barrier, and therefore can be done at much lower temperature. As a result, this method has gained great attentions^[57-60] in recent research activities on SWCNT growth because it simplifies the process flow to some extent, and widens the option for substrate material that can be chosen for growing the nanotubes. The CCVD is the method that has been devised to grow the SWCNT in this project, and I will discuss this method in more detail in the next section.

2.5.1 Experimental set up for SWCNT growth with CCVD

In contrast to electric arc-discharge or laser ablation, CCVD does not require any sublimation and de-sublimation stages. Hence, this aspect has resulted in lower temperature requirement for SWCNT growth. In addition, compared to other SWCNT growth methods, CCVD has been generally regarded as the most economical mean to produce SWCNT, because of its scalability and high throughput.

Figure 2.5(a) Schematic of a CCVD system (b) photograph of the CCVD system

Figure 2.5 above shows the schematic and photograph of a CCVD system devised in this project. In brief, the system consists of: (1) On Argon and H_2 mass-flow controller which act as the gas carrier, (2) an ethanol bubbler, which supply the carbon source, (3) a shuttle valve, which controls the flow direction of the carrier gases, (4) a furnace, (5) a quartz tube where the gas phase chemical reaction takes place, and (6) an exhaust pump to eliminate all the by-product from the reaction to the exhaust system.

In a typical growth process, the air in the quartz tube is first purged with the carrier gas mixture for a few minutes. This step is taken to make sure that no Oxygen is present in the quartz tube during the growth process, which involves Hydrogen and high temperature, for safety purposes. Afterward, the shuttle valve is set to re-route the carrier gas to the ethanol bubbler. Here, bubbles of carrier gases are injected into the ethanol, which upon bursting at the surface; the carrier gas is freed along with some ethanol vapor. The carrier/ethanol gas mixture then is transported to the furnace.

In general, the growth process in CCVD can be broken down into three steps: annealing, growth, and cooling. During the annealing step, only the carrier gas is transported into the quartz tube. At this step, the furnace is ramped up from 25 °C to 900 °C. The carbon feedstock gas mixture then is introduced into the furnace during the growth step. Finally, in the cooling step; the carbon feedstock gas is shut off, and the shuttle valve is reset, so that only the carrier gas is delivered to the furnace at this step. The furnace is ramped down to room temperature at the cooling step.

From a molecular perspective, the process of CCVD can be depicted like in Figure 2.6. A thin catalyst film of either Cobalt or Iron is first deposited on to a substrate, typically Silicon. During the annealing step, the catalyst film then breaks up to form nano-particles. In the growth stage, the carbon feedstock gas is introduced into the furnace. At this stage, some of the ethanol molecules that are attached on to the surface of the catalyst particle undergo a chemical reaction that frees up the carbon atoms.

The freed carbon atoms then are dissolved and form a solid-solution with the catalyst particle. However, after sometime, the concentration of carbon atoms in the particle would exceed the catalyst's solubility limit for carbon. When this critical concentration is reached, the carbon starts to precipitate and form the seed of the nanotubes.

Figure 2.6 Molecular mechanism of CCVD growth process

Upon formation of the nanotubes, the flow of the gas in the lateral direction causes the SWCNTs to bend over to follow the direction of the flow. Hence, in this aspect, the CCVD can be regarded as one manipulating method of SWCNT *in-situ* ^[61]. This ability over other growth methods has also make the CCVD one of the most preferred ways to grow SWCNT. The orientation shown in Figure 2.7 (a), unfortunately, is very difficult to obtain and often, a network of SWCNT as shown in Figure 2.7 (b) is obtained with the CCVD system.

Figure 2.7 (a) SWCNT grown with CCVD can align themselves with the direction of the gas flow. (b) Many times, however, a network of SWCNT is obtained.

The ease, high throughput, and scalability of this process have spurred many interests in the study of SWCNT growth with CCVD. For example, Zheng et al ^[62] reported a high efficiency growth of SWCNT with carbon monoxide precursor, hydrogen gas carrier, and two furnaces CCVD system. They stated that two furnaces are required in CCVD with carbon monoxide precursor, so that any impurities present in the commercial carbon monoxide is fully oxidized in the first furnace.

Baek et al ^[63] studied the formation of accompanying graphite that always takes place during CCVD growth of SWCNT. They argued that the formation of graphite on catalyst particles could happen at temperature approximately 700 °C, which is lower than the SWCNT growth temperature approximately 900 °C. The implication of their finding is that many of the catalyst particles during the SWCNT growth would be actually poisoned due to formation of the graphite at lower temperature.

Kukovitsky et al ^[64] argued in their publication that growth of SWCNT with CVD on metal surface, such as nickel, can be described as a complex multi-stepped process closely concerned with problems of heterogeneous catalysis, physical metallurgy, and phase transformations at the nano scale. They reported that the substrate surface condition can affect the activity of the catalyst particle, and the diffusion rate of carbon atoms. They further showed from X-ray diffraction data that there is a strong correlation between the catalytic activity and grain size of the nickel metal substrate. They also pointed out the possibility that surface roughness of a substrate, in general, affects the growth process of the SWCNT.

Burkh and Mitra ^[65] offered a more detailed analysis on the mechanism behind SWCNT growth with CCVD through a combination of experimental measurement and simulation result. They show that the reaction pathways that lead to the growth of SWCNT can be composed of a complex series of radical formation. Experimentally, they measured the length of the SWCNT located at different position in the CCVD tube. The differing length, they argue, would represent differing residence time of these radicals.

2.6 Chapter Summary

In this chapter I have discussed the electronic properties of SWCNTs: the E-k diagram of graphite, and how the one-dimensional nature of the nanotubes leads to zone-folding condition. Additionally, I have also discussed how the rolling direction in SWCNT influences its electrical behavior, in relation to the zone-folding condition.

I have also discussed CCVD in some details and show how this synthesis method fares

among other alternatives. CCVD as a method to synthesize SWCNT gains wide acceptance in the research field of nanotube, because it can give rise to SWCNT grow at relatively low temperature of 900 °C, compared with the typical 3000 °C for other techniques. In addition, CCVD is capable to produce high throughput of SWCNT per batch of growth process. The method also allows user to choose from wide-ranging carbon feedstock, including ethanol, which is relatively easy to obtain. The possibility to control growth direction through flow-alignment, or quartz template also adds the attractiveness of CCVD method. Having gaining an understanding of the physics behind SWCNT properties, and how to synthesize these nanomaterials, I shall now discuss about its device application in the next chapter.

Chapter 3 Liquid-Gated Carbon Nanotube Field-Effect Transistors

The unique electrical properties of SWCNTs has sparked a plethora of interesting potential applications ^[21, 28, 29, 46, 56, 66-68]. Specifically, in the field of biomedical devices, SWCNTs could play a significant role in terms of probing interaction between biomolecules at their native environment, and in real-time. The size similarity between SWCNTs and most biomolecules means that the two entities can interact rather well at the molecular level.

The hollow tubular structure ^[1] of the SWCNTs confines the electrical current to travel only on the surface of the nanotube. Correspondingly, the current would be very sensitive to changes from the nanotube surroundings. Hence, in principle, the interaction between the nanotube and the biomolecules could be monitored by measuring the electrical current that passes through the SWCNT.

In general, the electrical current measurement can be performed in either resistance or transistor format. The resistance measurement typically is easier to perform because it is influenced by only a small number of variables. However, this also means that resistance measurement can only give us limited information on the biomolecular interactions. Performing the current measurement on a transistor format is generally more complex because it is influenced by more variables. However, the large number of variables gives an opportunity to gain more information from the molecular interactions with the aid of

SWCNTs.

In this chapter, I shall discuss liquid-gated field-effect transistor (LGFET) as a biosensor. In particular, I shall discuss briefly about the physical working principles of the transistor, the role of capacitance, and simulation of the LGFET transistor behavior. The experimental set up, fabrication method and some basic characterization of the LGFET will be also described.

3.1 Band diagram of SWCNT in SWCNT FET

Tans et al ^[16] first demonstrated the fabrication of transistor with semi-conducting SWCNT which have a geometry as shown in Figure 3.1 (a). In brief, the device is constituted of a semi-conducting SWCNT flanked in between two metal electrodes, which are known in the transistor terminology as the source and drain electrodes. In a typical operation of the transistor, a small bias V_{ds} is applied across the SWCNT. This bias results in electrical current passing through the SWCNT, which is usually called the drain current I_{ds} . The conductance of the SWCNT, which is the native property of the nanotube, then can be calculated from dividing the I_{ds} with the V_{ds} .

The transistor behavior relies heavily on the modulation of this conductance by an act of a third electrode, which is usually called the gate electrode. In the case of Figure 3.1(a), the conducting Silicon substrate itself acts as the gate electrode. The SWCNT is separated from the gate electrode by a thin layer of dielectric material, such as Silicon dioxide. This dielectric materials prevents large current flowing from the gate electrode to the SWCNT, and it aids in capacitive-coupling the SWCNT with the gate electrode, and hence crucial

in the operation of the transistor.

Figure 3.1 (a) SWCNT FET in dry state configuration. (b) drain current as a function of gate voltage.

(c) band diagram of the SWCNT during operation of the transistor^[15].

Figure 3.1(b) shows how I_{ds} varies under the influence of the gate voltage V_g during the typical operation of the SWCNT FET in dry state. The transistor conducts well in the negative V_g territory, and completely shut off at positive V_g values. The value at which the transistor turns off, which in this case is approximately 2.5 V, is called the threshold voltage V_T .

To understand this transistor behavior, one should take a look at the band diagram of the SWCNT during the operation of the device, as shown in Figure 3.1(c). Whether or not the SWCNT conducts at a certain V_g depends on two separate issues: the position of the Fermi level at the contacts, and its position at the bulk of the SWCNT.

The position of the Fermi level at the contacts is determined primarily by the level work function of the metals, relative to that of the SWCNT. For the typical metals that is used (gold), the work function is higher than that of the SWCNT. This makes the Fermi level at the contacts lies slightly below the valence band of the SWCNT, and explains the p-type conduction at $V_g = 0$ V through the unoccupied valence states, as shown in the middle panel of Figure 3.1(c). Transistors with n-type behavior can be made by selecting metals which has work function smaller than the SWCNT, such as Calcium^[69]. Position of the Fermi level at the contacts has also been shown to be influenced by the presence of Oxygen in the ambient and the oxide^[70], and annealing the device in vacuum in gold-contacted SWCNT also has resulted in n-type behavior.

The position of the Fermi level at the bulk of the SWCNT is determined by the applied V_g . Applying negative V_g would push the electrons in the bulk of the SWCNT deeper into the valence band, as shown in the left panel in Figure 3.1(c). Plainly, this pushing would create ample of vacancies in the valence band, and hence accentuate the p-type conductance.

Inversely, applied positive V_g would induce electron enrichment in the SWCNT, which causes the Fermi level to raise closer to the edge of the valence band, and hence diminishes the number density of vacancies in the valence band. This diminution resulted in shut off of I_{ds} when the applied V_g is more positive than V_T .

If enough positive V_g is applied, the Fermi level can raise even across the band gap, and into the conduction band. In such event, a new conduction mechanism takes place whereby the electrons from the nearby contact valence band can jump into the

conduction band in the bulk of the SWCNT. Hence, at sufficiently high V_g , the SWCNT FET too exhibits n-type conduction. Presence of the barrier, however, makes the n-type conduction typically lower than that of p-type.

In dry-state measurement, the required V_g to observe this n-type conduction is prohibitively high that it could not be observed in most of the cases. This is a direct consequence from the fact that Silicon dioxide has a considerably low dielectric constant ($\epsilon_r \sim 3.9$), which results in poor capacitance coupling between the gate electrode, and the nanotube film.

On the contrary, aqueous solution typically has a very high dielectric constant of approximately 80. This high dielectric constant results in most of the bias applied in the gate electrode actually appears on the SWCNT. Hence, n-type conduction can be observed in the SWCNT LGFET, as it will be seen in this thesis.

3.2 Capacitance in SWCNT FET

Recalling that in the typical transistor architecture the SWCNT is separated from the gate electrode by a thin layer of dielectric material, expectedly, the extent of the influence of gate electrode on the SWCNT is decided by the thickness, and the dielectric constant of the dielectric materials. Or, generally, the influence is decided by the capacitance coupling between the gate electrode and the SWCNT.

In SWCNT FET, there are two components of this capacitance: the classical electrostatic capacitance between the gate and the SWCNT, C_E , which depends on the thickness and

the dielectric constant of the dielectric materials; and the quantum capacitance, C_Q , which depends on the SWCNT density of states (some energy is required to add or subtract charges to the next available state). Electrically, these two capacitances can be imagined as being connected in series with each other. As such the resultant capacitance is described by

Equation 3.1

$$\frac{1}{C_{total}} = \frac{1}{C_Q} + \frac{1}{C_E}$$

Strictly speaking, the metal gate electrode actually also has its own share of C_Q . However, since its dimension typically is much larger than the SWCNT, its C_Q is much larger than the SWCNT. Since the total capacitance is decided by the smallest capacitance, as revealed in Equation 3.1, the contribution from the C_Q of the metal electrode can be neglected.

Oullette^[15] showed that the typical value of C_E from a SWCNT separated from the metal gate electrode with a 200-nm Silicon dioxide is approximately $C_E = 3 \times 10^{-17}$ F/ μm ; while the typical value of C_Q in a SWCNT is approximately $C_Q = 4 \times 10^{-16}$ F/ μm . Hence, the C_E dominates the total capacitance in dry-state measurement.

Figure 3.2 (a) shows the typical set up in LGFET experiment. Here it is shown that the aqueous solution has replaced the dielectric in the dry-state transistor, and Figure 3.2 (b) shows the potential distribution in the droplet of the solution upon application of gate and source/drain bias. As noted, the potential in the bulk of the droplet is dictated primarily

by the gate bias, and the source/drain bias affects only the potential close to the junction of the drain electrode (whereby the bias is usually applied).

Figure 3.2 (a) typical set up of LGFET, and (b) potential distribution in the droplet of water upon application of gate and source/drain bias. Simulation condition: V_g -0.7 V, V_{DS} 0.01 V, ϵ_r 80.

Figure 3.2 also implies that the C_E in LGFET is not as simple as that of the dry-state. However, for rough calculation, it can be assumed that the representative C_E for LGFET is that of parallel-plate capacitor with thickness equivalent to the radius of the droplet. In the typical experiment, the volume of the droplet is about 50 μl . This volume is equivalent with a radius of approximately 288 μm .

In addition, in the typical experiment, the SWCNTs in the device are in the form of a random network. Hence, one can treat the SWCNT film as though it is a continuous film with area typically in the order of $3.5 \times 10^5 \mu\text{m}^2$. Hence, the typical C_E in the LGFET can

be calculated.

Equation 3.2

$$C_E = \epsilon \epsilon_r \frac{A}{d}$$

Equation 3.3

$$C_E = (8.85 \times 10^{-14}) (78) \frac{(3.5 \times 10^5) (1 \times 10^{-8})}{(2.88 \times 10^{-4})} = 8.38 \times 10^{-11} \text{ F}$$

If I normalize it with the typical length of the LGFET (approximately 100 μm), I would obtain the C_E value approximately $8.38 \times 10^{-13} \text{ F}/\mu\text{m}$. The calculation result from Equation 3.3 tells that in liquid-gated measurement, the C_E component dominates the total capacitance. Note as well that the capacitance in liquid-gated set up is higher than that of dry-state measurement. This is the direct consequence of the fact that aqueous solution has higher dielectric constant than Silicon dioxide. The difference in C_E between liquid-gated and dry-state that is obtained here, however, is not as big that it supposed to be. This smaller difference probably is because I have approximated the capacitor with that of parallel-plate capacitor, which under-estimate the total available area in the liquid-gated set up.

3.3 Simulation of LGFET

In section 3.1 I have discussed how the band diagram in the SWCNT influenced by applied V_g to give rise the transistor behavior, and in section 3.2 the capacitance component in a LGFET device have been explained. In this section, the integration

between these two concepts shall be described in a set of equations which defines the behavior of a SWCNT FET device.

The model that explains the SWCNT FET device can be derived in two stages. The first stage describes the set equation that explains ballistic (perfect) conduction in the SWCNT, whereby the transistor behavior borne out from the Schottky-barrier. Defects and barrier due to imperfection, or SWCNT/SWCNT junction found in SWCNT film can be modeled by introducing classical Drude-mode transport in the bulk part of the SWCNT, which is done in the second stage.

For simplicity, the model explained herein is based on a simple SWCNT FET architecture which is composed of three electrodes, one SWCNT, and a dielectric layer. Plainly, the model is a rather simplistic representative of the real LGFET device. However, results borne out from this model can act as a qualitative guide of what could be expected from the biosensing experiments described in later chapters.

More specifically, the aim of the model is to find out beforehand (roughly) the outcome of many possible interactions that could take place between the SWCNT and the biomolecules.

Figure 3.1 (b) shows that the bands diagram in the SWCNT bends upward (downward) when a negative (positive) V_g is applied. Jimenez et al.^[71] calculated analytically the shape of the band diagram upon influence by the V_g . They noted that the bottom of the conduction band profile at the source and drain regions can be stated as

Equation 3.4

$$E_S = \varphi_{SB} - q(V_{GS} - V_{FB}) \left(1 - e^{\frac{-2x}{t_{dielectric}}} \right)$$

Equation 3.5

$$E_D = (\varphi_{SB} - qV_{SD}) - q(V_{GS} - V_{FB}) \left(1 - e^{\frac{-2(x-L)}{t_{dielectric}}} \right)$$

Where φ_{SB} is the Schottky barrier, q is the elemental charge; V_{SD} is the source/drain bias, V_G is the gate bias, V_{FB} is the flat-band bias, x is the distance from the Source electrode, L is the length of the SWCNT, and $t_{dielectric}$ is the dielectric thickness (or Debye length in the case of LGFET, as shall be explored in the next few chapters). The valence band has the same profile with the conduction band, but downshifted by a value equal to the band gap of the SWCNT.

The electrical current through this FET architecture then is calculated according through Landauer-Büttiker formalism, which assumes ballistic conduction mode in the middle-section of the SWCNT.

Equation 3.6

$$I_{ds} = \frac{4q}{h} \sum_n \int_{-E_1}^{E_2} T(E) (f_s(E) - f_d(E - qV_{ds})) dE$$

Where h is the Planck constant, $T(E)$ is transmission probability, and $f(E)$ is the Fermi-Dirac distribution. Equation 3.6 accounts for the spin degeneracy of the injected charge

carriers. To keep the calculation simple, the summation in Equation 3.6 is performed only on the first channel. The integration limits E_1 and E_2 are the limits on energy values as defined by the first Brillouin-zone, close the global minima.

As noted from Equation 3.6, the term $T(E)$ plays a critical role in the working of the SWCNT FET. Following Jimenez et al^[71], the $T(E)$ through single Schottky-barrier is calculated through Wentzel-Kramers-Brillouin (WKB) approximation, which is given by:

Equation 3.7

$$T = \exp\left(-2 \int_{z_i}^{z_f} k_x(x) dx\right)$$

where k_x is the carrier wave number in x -direction (along the length of the SWCNT), and the integration limit z_i and z_f are the classical turning point, whereby the band profile starts to flatten. A simple calculation would reveal that these points typically are located at $t_{ins}/2$.

The carrier wave number is linked to the energy level through E - k relationship Equation 2.5, which in the linear regime can be approximated with:

Equation 3.8

$$E - E_{S(orD)} = \pm \frac{3d}{2} \gamma_{\pi\pi} \sqrt{k_n^2 + k_z^2}$$

where $d=0.142 \text{ nm}$ is the carbon-carbon bond distance in the SWCNT, $\gamma_{\pi\pi} = 2.97 \text{ eV}$ is the nearest-neighbor interaction, k_n is the component of the wave number in the radial

direction. The ‘+’ and ‘-’ signs are referred to the conduction and valence band, respectively. For a SWCNT with chiral vector (n_1, n_2) ; the perpendicular component k_n is given by:

Equation 3.9

$$k_n = \frac{|3n - n_1 + n_2|}{3R}$$

Where R is the SWCNT radius:

Equation 3.10

$$R = \frac{d}{2\pi} \sqrt{3(n_1^2 + n_2^2 + n_1 n_2)}$$

Substituting Equation 3.8 and Equation 3.10 to

Equation 3.7, and rearrange, and the transmission coefficient $T(E)$ through a single Schottky-barrier can be stated as:

Equation 3.11

$$T_n(E) = \exp \left(- \int_{z_i}^{z_f} \frac{2E_g}{3d\gamma_{\pi\pi}} \sqrt{\left(\frac{3d\gamma_{\pi\pi} k_n}{E_g} \right)^2 - \left(\frac{E - E_{S(orD)}(x)}{\frac{E_g}{2}} \right)^2} dx \right)$$

Whereby E_g is the band gap in the SWCNT, which is related to the radii of the SWCNT through relationship:

Equation 3.12

$$E_g = \frac{\gamma_{\pi\pi} d}{R}$$

Equation 3.11 must be solved numerically for each energy-level to obtain the drain current I_{ds} . The same calculation is performed throughout the selected range of V_g to obtain the transfer characteristic ($I_{ds} - V_g$) performance of the SWCNT FET.

Landauer-Büttiker formalism in Equation 3.6 gives us the I_{ds} - V_g for the case of ballistic transport. This assumption leads to over-estimation of the real current in the device, which typically works in the diffusive regime, whereby Drude model (Equation 2.10 to Equation 2.12) thrives.

Following Heller et al^[23], in the presence of Schottky-barrier conduction mode, and diffusive mode; the resultant conductance can be depicted as two conductances (one for each mode of conduction), connected in series:

Equation 3.13

$$\frac{1}{G_R} = \frac{1}{G_{Schottky}} + \frac{1}{G_{Drude}}$$

Hence, the resultant drain current will be determined by the lower conductance.

From this model, it becomes clear that there are four possible scenarios whereby SWCNT can interact with the biomolecules. They are: (1) Schottky barrier modulation, (2) charge modulation, (3) capacitance modulation, and (4) mobility modulation. Figure 3.3 shows

how each of this interaction mechanism leaves a certain signature in the transfer characteristic of the SWCNT FET ^[23].

In the case of electrostatic gating modulation, when positive biomolecules is adsorbed in the proximity of the nanotubes, an induced negative charge layer is formed on the surface of the SWCNT, which hinder the passage of holes in the SWCNT, and in turn reduce the I_{ds} , at all V_g level in the liner region. This reduction, correspondingly, can also be regarded as a V_t shift if it is looked from the V_g perspective. Likewise, the reverse mechanism happens when negative biomolecules attaches on to the surface of the SWCNT.

Schottky barrier modulation in essence also shows similar feature to that of electrostatic gating. Nonetheless, the extend of the V_t shift in Schottky barrier modulation is not that great in normal situation. This is because in order to greatly disturb the Schottky barrier, the biomolecules has to push (or pull) a large number of electrons in the metal electrodes to significantly modulate its Fermi level. Recalling that most biomolecules have only weak charge on their surface, a great Schottky barrier modulation, hence, seems unlikely. Nonetheless, a biomolecule still can change the Schottky barrier to a limited extent, and such shift is observed most clearly in the extremes values of the I_{ds} - V_g as shown in Figure 3.3 (b). Here it is shown that when the Schottky barrier is increased, the I_{ds} in negative V_g are reduced, but the I_{ds} in positive V_g range are increased, similar to that of electrostatic gating effect, only with a limited shift. Alternatively, such limited shift in the V_T can be regarded as a 'tilt' in the I_{DS} - V_G curve.

Figure 3.3 Four possible scenarios whereby biomolecules can interact with SWCNT: (a) charge modulation, (b) Schottky barrier modulation, (c) capacitance modulation, and (d) mobility modulation. Simulation results as calculated from Landauer – Büttiker formalism, taking into account the effect of classical Drude’s model. Black: the simulated I_{DS} - V_G response *before* the biomolecular interaction. Red: the simulated I_{DS} - V_G response *after* the biomolecular interaction.

Capacitance and mobility modulation, on another hand, influence the saturation current (I_{sat}) and the slope of the I_{ds} - V_g curve, respectively. Specifically, Figure 3.3 shows that reduction in mobility causes a decrease in the slope, while diminution of capacitance

leads to a decrease in I_{sat} in the $I_{\text{ds}}-V_{\text{g}}$ curve. The physical reason behind this phenomenon can be explained as follows.

Capacitance can be regarded as a measure of coupling between the gate electrode and the SWCNT. A higher capacitance usually results in a better coupling between the electrodes and the nanotubes, which results in the charge carriers driven to higher I_{sat} value, and lower capacitance results in lower I_{sat} values.

Mobility, on another hand, is a measure of the ease of charge carriers to pass through a material. Hence, materials with high mobility usually display larger I_{DS} swing in response to a V_{G} bias, due to its nature for being more responsive. Conversely, if this mobility is reduced because of scattering, for example, the I_{DS} become less responsive toward V_{G} . Therefore, it is perhaps unsurprising that the effect of mobility is most clearly observed in the slope of the $I_{\text{DS}}-V_{\text{G}}$ curve.

Thus, Figure 3.3 implies that analysis of how the $I_{\text{ds}}-V_{\text{g}}$ curve changes its shape and position could reveal important information on how the biomolecules actually interact with the SWCNT. In particular, the following parameters are important in this type of analysis: (1) threshold voltage, (2) sub-threshold slope, and (3) saturation current. In addition, measurement of I_{ds} as a function of time, the so-called kinetic measurement, could reveal how this interaction actually evolves over time.

Hence, it is quite clear that SWCNT FET in liquid-gated configuration offers an interesting opportunity in that it can offer much information regarding how biomolecules interact with the device, which could be useful as both for sensing application as well as

for fundamental studies. On top of that, the additional capability of SWNT FET to do real-time measurement in kinetic mode gives SWCNT FET a leverage to monitor this interaction as and when the event happens, and how it evolves.

3.4 Experimental set up with LGFET

In the previous section, the set of equations that govern the working principle behind LGFET have been discussed. In this section, the focus will be predominantly on the set up employed to perform the electrical measurement conducted in liquid environment.

Figure 3.4 Schematic of the electrical measurement set up for LGFET. Modules of program made in LabVIEW 7.01 are used to collect, plot, and save readings from the electronic instruments.

All LGFET measurements are conducted with the aid of a home-made system, which diagrammatically is shown in Figure 3.4. In brief, the system consists of the following

constituents: (1) one reference electrode that act as a gate electrode, (2) one function generator to apply voltage to the reference electrode (V_g), (3) one oscilloscope to monitor the gate voltage, (4) the liquid sample, (5) the carbon nanotube field-effect transistor, which in itself consists of: (i) carbon nanotube film, (ii) metal electrodes which act as source/drain electrode, and (iii) a substrate; (6) one voltage source connected to the source/drain electrode (V_{ds}), (7) one pico-ammeter to measure the source/drain current passing through the transistor (I_{ds}), and (8) one personal computer to acquire, record and plot the data.

In principle, the working mechanism behind this system is similar to that of normal probe station, except that in this system, the gate probe has been connected to a reference electrode. Ouellette ^[15] has shown that the reference electrode is critical to keep variation from interfacial potential to minimum. This is because the design of the reference electrode allows minimum change in the solution composition around the electrode, which helps to stabilize the interfacial potential. In contrast, if ordinary metals, such as: platinum, titanium, etc; are used as the gate electrode, variation in the chemical composition in the solution in proximity to the metal electrode would induce uncontrolled variation of the interfacial potential. Clearly, such an uncontrolled variation is undesirable from the experimental perspective.

In brief, the liquid solution in LGFET replaces the function of dielectric in the ordinary MOSFET. A function generator (*TTi TG1304*) applies the V_g bias on to the Ag/AgCl reference electrode (*World Precision Instrument*, isoplast[®] polymer coating, diameter 2 mm, length 12 cm), which is continuously monitored by an oscilloscope (*Agilent*

DSO3062A). The capacitance coupling between the reference electrode and the SWCNT channel, mediated by the aqueous solution; causes conductance modulation in the SWCNT (*cheaptubesinc*) channel. In parallel, a V_{ds} is also applied across the source-drain electrode in the device and the I_{ds} is measured with a pico-ampere meter. In this set up, the V_{ds} bias and the pico-ampere meter functionality are provided by a single instrument *Keithley 6427*. The signal that comes from the interaction between the device and biomolecules is observed either through I_{ds} - V_g measurement, or kinetic measurement. Data points from the measurement are collected with several programs written in *LabVIEW 7.10*. All the programs are running on a Windows XP operating system, and have been tested with standard transistors prior to testing with the LGFET.

In order to simplify the program architecture, and to breakdown the task into manageable packages, the program is broken down into several modules: (1) liquid gating, (2) sync liquid gating, (3) $I_d V_d$, and (4) converter. The module *liquid gating* basically is the most important in the experimental set up, for this is the module which actually establishes a communication channel between the personal computer, and all other electrical instruments. In this module, the V_{ds} , V_g and I_{ds} are monitored as a function of time, and the I_{ds} - V_g is plotted in real-time as the V_g is swept across a predefined values. In addition, this module also calculates the conductance of the SWCNT channel by dividing the I_{ds} with V_{ds} , at all V_g levels. In normal operation, the V_g usually is swept across pre-defined values, while keeping the V_{ds} constant. Alternatively, in kinetic mode, the V_g and V_{ds} can be set constant throughout the experiment, while reading the I_{ds} as a function of time. Figure 3.5 shows the front panel of *liquid gating* module.

Figure 3.5 A snapshot of front panel of *liquid gating* module. This module establishes the communication channel between the personal computers and all the electrical instruments.

Sync liquid gating module is built to help analyzing data gathered by *liquid gating* module in real time. Technically, the *sync liquid gating* module has a master-slave relationship with the *liquid gating* module in that the later acts like the master, while the *sync liquid gating* acts as the slave. That is, the *sync liquid gating* module receives (or synchronize) its data from the *liquid gating* module. In this module, the data points received from the *liquid gating* are fed to a set of commands that helps to perform several analyses, which include: (1) curve fitting for I_{ds} - V_g plot, (2) saturation current, (3) first derivative of I_{ds} - V_g curve, (4) threshold potential V_T , and (5) R^2 value from the curve fitting procedure. These analyses are performed every time the V_g completes a single cycle, and the results are pooled in the memory. As such, *sync liquid gating* helps to analyze the data as and when the experiment is running. Figure 3.6 shows the front panel

of *sync liquid gating* module.

Figure 3.6 A snapshot of front panel of *sync liquid gating* module. This module helps to analyze the data gathered by *liquid gating* in real-time.

The third module, *IdVd*, also establish a communication channel between the personal computer, and all other electrical instruments. However, this module only performs I_{ds} - V_{ds} measurement. In normal operation, this module is operated with V_g held at a constant value, while sweeping V_{ds} through pre-defined values. The module is an excellent additional tool to confirm the transistor behavior of the device. Figure 3.7 shows the front panel of *IdVd* module.

Figure 3.8 A snapshot of front panel of *converter* module. This module helps to design the electrical experiment by converting three units which are normally used to define a sweeping rate. Sweeping rate is important because it affects the magnitude of the leakage current which passes through the liquid sample.

3.5 Fabrication methods of LGFET

In general, there are two types of LGFET: silicon-substrate LGFET (*ss*-LGFET), and flexible-laminated LGFET (*fl*-LGFET) ^[72], which was formulated during the course of this project. Expectedly, the fabrication methods for these two types of devices are rather different.

Figure 3.9 shows the fabrication workflow for *ss*-LGFET. The fabrication process for *ss*-LGFET starts with growth of SWCNT with CCVD method as described previously. Afterward, gold source-drain electrodes were deposited on top of the SWCNT by using electron-beam evaporation. The partially-completed assembly formed the device for biosensing studies.

To perform the sensing studies, a droplet of liquid-sample was added on top of the

Silicon/SWCNT/gold assembly. The liquid-sample in this case replaces the dielectric materials in ordinary transistors. A gate bias then was applied to the liquid sample through insertion of a reference electrode. The biomolecular interaction then was studied by monitoring the I_D - V_G curve, or through kinetic measurements.

Figure 3.9 Fabrication process of ss-LGFET. (1) Growth of SWCNT with CCVD with Silicon substrate, (2) deposition of gold electrode with electron-beam evaporation, (3) liquid sample is applied on top of the gold/SWCNT assembly, (4) a gate bias is applied through insertion of a reference electrode to the liquid sample.

The ss-LGFET is a common type of LGFET reported in the literatures^[7, 73, 74]. While it can perform well in the liquid-environment, the architecture of the device involves tedious fabrication process such as in situ growth or deposition of SWCNT and patterning of gold electrodes by photolithographic techniques. Involvement of high temperature during the SWCNT growth delimits the process to few choices of substrate

materials, with Silicon being the most commonly used. Furthermore, the fabrication process typically takes several hours to completion. This is because of SWCNT growth usually involves a good amount of time to attain fine stability in the growth temperature, or stable background vacuum pressure in the deposition process.

The device architecture of *fl*-LGFET * ^[14], on another hand, does not suffer from this drawbacks, while still displays good transistor performance for sensing biomolecular interaction in aqueous media. Figure 3.10 shows the fabrication workflow of *fl*-LGFET.

The fabrication process starts by preparing three items: (1) featureless Poly – (dimethyl siloxane) (PDMS) slab, (2) Molded PDMS slab to infuse microchannel features, and (3) SWCNT aqueous dispersion. A silicon mold of the desired microchannel was prepared by standard photolithography methods. The mold was then used as a master to form by casting a microchannel into the polymer film. Afterward, two PDMS films are prepared. One of the PDMS is casted on to the silicon mold, while the other is casted in a blank Petri dish. The result of this step is two PDMS slabs: one with microchannel (Figure 3.10 (1)(a)) and another one that is featureless (Figure 3.10 (1) (b)).

Concurrently, a carboxylated SWCNT (c-SWCNT) aqueous dispersion (0.5 mg ml^{-1}) in 10 % sodium dodecyl benzene sulfonate (SDDBS) surfactant is prepared. The resulting SWCNT solution is then filtered on an alumina filter (*Whatman*, $0.1 \text{ } \mu\text{m}$) and flushed with copious amount of distilled water until the surfactant is completely removed, as

* Patented in United States of America, Application # 61/136,626

indicated by the absence of bubbles in the filtrate. The result of this filtration step is a SWCNT film on the alumina filter. Typically, a larger volume (approximately 500 μl) of c-SWCNT solution is used to fabricate source-drain electrode than electrical channel (approximately 100 μl). The larger volume would results in a denser SWCNT film, and hence behaves more like metallic material, than channel region^[75-77].

Figure 3.10 Fabrication workflow of *fl*-LGFET. (1) (a) molded microchannel and (b) blank PDMS are prepared. (2) SWCNT film is then stamped on to the (a) microchannel and (b) blank PDMS. (3) Laminate the two PDMS together to form a complete working device. (4) liquid-sample is applied through the micro-channel and a gate bias is applied to the liquid-sample through insertion of a reference electrode.

The SWCNT film is then stamped onto the PDMS (Figure 3.10 (2) (a) and (b)) to form

source-drain electrode and electrical channel in the *fl*-LGFET. Subsequently, the two PDMS slabs are laminated to complete the device (Figure 3.10 (3)). The liquid-sample is introduced into the microchannel through suction with a syringe suck-head, and the gate bias was applied to the liquid sample through insertion of a reference electrode (Figure 3.10 (4)).

3.6 Basic characterization of LGFET

In this section, the transistor behavior of *ss*- and *fl*-LGFET shall be discussed, as measured by the electrical system in Figure 3.4. This verification is particularly important before I move on to discussing about probing biomolecular processes in the next chapter, because it will give a confirmation, and to establish the characteristics of the LGFETs before sensing is performed. Specifically, the transistor is characterized by measuring the I_{DS} - V_G and I_{DS} - V_{DS} plot and monitoring the leakage current (I_{leak}) that would indicate of the typical operating range for the I_{DS} .

Figure 3.11 (a) I_{DS} - V_G curve of LGFET (V_{DS} 10 mV). The I_{DS} increases as V_G becomes more negative, indicative of p-type behavior from the SWCNT. (b) I_{DS} - V_{DS} curve of LGFET at different V_G bias. The I_{DS} increases as V_{DS} is increased and V_G becomes more negative, again indicating p-type behavior from the SWCNT. (c) Kinetic measurements featuring I_{DS} and I_{leak} . V_G and V_{DS} are set at -500 and 10 mV, in that order. The I_{DS} in LGFET typically is in the order of $0.5 \mu\text{A}$, while I_{leak} typically is in the order of 2 nA.

Figure 3.11 (a) shows the typical I_{DS} - V_G curve obtained from LGFET. As noted, the I_{DS} increase as V_G bias becomes more negative, indicative of p-type behavior from the SWCNT^[15, 41, 48, 50, 71, 78]. The sub-threshold swing, obtained from I_{DS} - V_G is

approximately 2463.054 mV/decade, and ON/OFF ratio of only 1.63. These values are relatively poor in comparison with commercial transistor performance used in personal computers^[79, 80], whose sub-threshold swing and ON/OFF ratio can reach to 60 mV/decade and $>10^5$, respectively. However, since the LGFETs developed herein are not used for switching, these values are sufficient for biosensing applications.

Furthermore, by using transistor action equation

Equation 3.14

$$I_{DS} = \frac{W}{L} \mu C_{liquid} \frac{(V_G - V_T)^2}{2}$$

, the mobility of charge carrier in LGFET through the slope in the I_{DS} - V_G curve, as dictated by the equation below:

Equation 3.15

$$\frac{dI_{DS}}{dV_G} = \frac{W}{L} \mu C_{liquid} (V_G - V_T)$$

Equation 3.16

$$\mu = \frac{1}{\left(\frac{W}{L}\right) C_{liquid} (V_G - V_T)} \frac{dI_{DS}}{dV_G}$$

, where I_{DS} and V_G mean as usual, and W , L , C_{liquid} , μ , and V_T denote the width, and length of the electrical channel, respectively; capacitance in the LGFET per unit area, mobility,

and threshold potential, in that order.

The W and L values in the LGFET were set at 400 and 40 μm , respectively. Taking conservative value of C_{liquid} in the order of $5.24 \times 10^{-6} \text{ F/cm}^2$ ^[72], $(V_G - V_T)$ of approximately 200 mV, and $\frac{dI_{DS}}{dV_G} 1.851 \times 10^{-6} \text{ A/V}$, the μ in the LGFET can be calculated using Equation 3.16 to arrive at value: $17.66 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. This μ value is much lower than that of charge carriers in Silicon (approximately $1400 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$), which may be caused by the fact that the LGFET is made up from a network of SWCNTs. The random arrangement of the nanotubes in the network can be expected to degrade the overall mobility of the charge carriers in the LGFET. Furthermore, the low mobility value found in LGFET also indicates that the charge carrier in the device conducts electrical current through diffusive transport mechanism^[15, 81] as opposed to ballistic transport mechanism^[16, 46].

Confirmation of the transistor action is also displayed in Figure 3.11 (b), which shows I_{DS} - V_{DS} curve from the LGFET. In this case, the I_{DS} are observed to increase as V_{DS} is increased, and V_G becomes more negative; again, indicative of p-type behavior from the SWCNT. Overall, the I_{DS} - V_{DS} behavior as observed in Figure 3.11 (b) follows a transistor action in linear region:

Equation 3.17

$$I_{DS} = \frac{W}{L} \mu C_{liquid} \left(V_G - \frac{V_T}{2} \right) V_{DS}$$

, and corroborates the I_{DS} - V_G result in Figure 3.11 (a).

Additionally, it is also useful to characterize the I_{leak} in the device through kinetic measurement with V_{DS} applied is set to zero. The knowledge of I_{leak} magnitude is helpful, as it would assist to ascertain whether or not the expected signal comes from the I_{DS} , and to establish the expected I_{DS} drop that can be tolerated. Figure 3.11 (c) displays the magnitude of I_{DS} and I_{leak} as measured during a kinetic measurement. It is noted that the magnitude of I_{DS} is at least three orders of magnitude higher than that of I_{leak} , and hence the contribution of I_{leak} in the analysis can be safely neglected.

3.7 Chapter Summary

In this chapter, the working principle behind SWCNT FET and LGFET have been addressed. In a typical architecture, SWCNT FET is consisted of two metal source and drain electrodes, which flanks a semi-conducting material in between. The transistor action in SWCNT FET comes about from bending of band diagram in the SWCNT upon application of a V_{G} bias. Ideally, this mechanism should impart bipolar behavior on to the SWCNT. However, in practice, adsorption of Oxygen usually shifts the Fermi level closer toward the valence band, and hence suppresses the n-type behavior in most of the cases.

Literatures have shown that the Schottky contact effect can be captured nicely with Landauer-Buttiker formalism. Solving the equation reveals that the interaction between biomolecules and SWCNT should shows a signature pattern in the change of $I_{\text{DS}}-V_{\text{G}}$ curve. Briefly, electrostatic gating typically produces a shift in the $I_{\text{DS}}-V_{\text{G}}$ curve toward positive or negative bias direction, depending on the overall charge that surrounds the

SWCNT. Schottky barrier changes, on the other hand, resulting in a decrease in I_{DS} at negative V_G bias, while increasing I_{DS} at positive V_G bias. Furthermore, a change in the capacitance usually is typified by modulation in the slope of the I_{DS} - V_G . A mobility change is usually characterized by a decrease in I_{DS} at both negative and positive V_G bias.

The electrical measurement system set up to perform all the measurements was also discussed. In short, the system requires two voltage source and one pico-ampere meter. One of the voltage sources is used to supply the V_G bias, and another was used to apply the V_{DS} . The pico-ampere meter was employed to measure the I_{DS} . All these electrical instruments were then synchronized to a personal computer with a home-made program.

Furthermore, the fabrication method of two types of LGFET: *ss*- and *fl*-LGFET; have also been explained. The advantage of *ss*-LGFET is that since the SWCNT are grown directly from CCVD process, it does not contain any surfactant residue. On the other hand, the plus point from *fl*-LGFET is the integration of microfluidic channel in the design, which gives access to a better control to the liquid-sample. Additionally, *fl*-LGFET fabrication process is much simpler and faster than that of *ss*-LGFET.

Basic characterization of the *ss*- and *fl*-LGFET was also then discussed. The I_{DS} - V_G curve and I_{DS} - V_{DS} curve both show that *ss*- and *fl*-LGFET exhibit a p-type behavior, similar to previous reports. Furthermore, analysis from the I_{DS} - V_G curve reveals that the mobility in both *ss*- and *fl*-LGFET is significantly lower than that of Silicon, which may be attributed to the random network nanotube arrangement in the device.

Chapter 4 Protein Conformational Changes Induced by Interactions with Carbon Nanotubes and its Implications on Biosensing¹

The unique electronic properties of single-walled carbon nanotubes (SWCNTs) and their size similarity with most biomolecules ^[82] have resulted in numerous applications in biology-related fields, such as: biosensors ^[23, 82], biomedical implants ^[83], and supports for adhesion of liposaccharides to mimic cell membranes ^[13] amongst others. To this end, the surface functionalization and conjugation of biomolecules to SWCNTs is imperative to render the nanotubes compatible with the aqueous biological milieu ^[18], or to enhance essential selectivity in biosensors ^[68]. This surface functionalization could however influence the toxicity and biocompatibility of SWCNTs ^[84].

The conformational changes that result from protein-SWCNT interaction have been reported ^[85]; however, its impact on the conductance of the nanotubes is still obscure and warrants further attention. In this chapter, the interaction between BSA, a model protein, with pristine and carboxylated SWCNT will be elaborated. Mapping of conformational changes of proteins, as characterized by fluorescence and circular dichroism (CD) are

¹ The work presented in this chapter has been published: *Protein/carbon nanotube interaction: the effect of carboxylic groups on conformational and conductance changes* • 2009 • **Appl. Phys. Lett.** • Vol 95, p 073704

coupled here with real-time liquid-gated field-effect transistor (LGFET) study to document the effect of protein-SWCNTs interaction on the change in conductance.

4.1 Experimental details

Pristine SWCNTs were used as purchased from *Carbon Solution, Inc.* Carboxylated SWCNTs were synthesized from pristine nanotubes through acid treatment ^[86]. The physisorption of the BSA on to the nanotubes was characterized with an atomic force microscopy (AFM) (*Nanoscope IIIa*, tapping mode in ambient environment) on bare poly (dimethyl siloxane) substrate. The substrate material is chosen because its typical roughness (root-mean-square 0.772 nm) is not exceeding the diameter of the SWCNTs (2 – 4 nm).

Pristine and carboxylated SWCNTs were incubated in aqueous BSA solution (100 $\mu\text{g ml}^{-1}$, 2 h) with concentrations ranging from 0, 0.01, 0.02, 0.025, 0.03, and 0.05 mg ml^{-1} (or 0, 1.74, 3.74, 4.34, 5.21, and 8.68×10^{-9} M, respectively), prepared in phosphate buffer (PB) solution with pH 7. The stability of the conjugate, as well as changes in the BSA structure, was monitored by fluorescence spectroscopy (*Perkin Elmer LS 50B*, excitation: 290 nm). Circular dichroism (CD) spectroscopy (*Jasco-J810*, 0.1 cm path length quartz cell) was also employed to study the BSA conformation upon interaction with SWCNT. Percentage of α -helix content in the BSA was calculated from the resulting CD spectra as detailed elsewhere ^[87].

Conductance changes induced by BSA-SWCNT interaction were studied in real-time with a liquid-gated field-effect transistor (LGFET) ^[72]. The LGFET detects changes in

SWCNT conductance caused by variation in electrostatic potential resulting from SWCNT-biomolecular interactions. In particular, presence of positive / negative charges usually is associated with a decrease / increase in the SWCNT conductance.

In order to investigate the BSA/nanotube interaction in more detail from the molecular perspective, a molecular dynamic simulation has been performed. The simulation in this regard is necessary to highlight some information unavailable to us from the experiments, and yet may be useful in explaining the observed conductance modulation, and conformational changes as observed from the LGFET and spectroscopic measurements, respectively.

In simulating the BSA-SWCNT interaction with *ArgusLab 4.0.1*, the native conformation of the BSA structure was obtained from SAM-T08 server ^[88]. The geometry of SWCNT was built in *Arguslab 4.0.1* and optimized through universal force field (UFF) molecular mechanics Hamiltonian. Optimized geometry was obtained when the difference between two calculated Hamiltonian converges to a value less than 0.1 kcal mol⁻¹. The best interaction configuration was searched through *GADock* engine, which performs minimization of the binding free-energy, and the extent of the interaction was quantified by measuring the size of the binding pocket, as well as calculating the dipole moment of the BSA, before and after the interaction. The sensitivity of the searching algorithm was tested by repeating the simulation for three times varying both the number of iteration: 200, and 1000; and the convergence limit: 10⁻¹⁰ and 10⁻¹ kcal mol⁻¹. The average and the standard deviation of the binding pocket size and the dipole moment from the triplicate simulations were then calculated.

4.2 Results and Discussion

Proteins, such as BSA, and SWCNT are considered macromolecules which can be easily identified with AFM with the sparse linear features corresponding to tubular structure of SWCNTs (Figure 4.1 (a)). Physisorption of BSA on nanotubes is evident from globular features, attributable directly to the BSA that resides on top of the linear features (Figure 4.1 (b)).

Figure 4.1 (a) Atomic force micrograph of (a) bare SWCNT and (b) BSA-SWCNT conjugate stabilized through physisorption. The stabilizing force which contributes to physisorption is primarily weak hydrophobic interaction between the nanotube and the BSA.

This physisorption is typically stabilized by a weak hydrophobic interaction between BSA and SWCNTs that may be examined through intrinsic fluorescence of tryptophan

residue of the protein ^[87]. The opening of the BSA conformation exposes the tryptophan residue closer to the SWCNT and leads to reduction in the fluorescence intensity due to an efficient energy transfer between the two molecules. The change in the fluorescence spectra (Figure 4.2 (a) and (b)) is more apparent in the case of carboxylated than pristine SWCNT. Furthermore, since tryptophan fluorescence intensity (F) is related to the SWCNT concentration $[SWCNT]$, the dissociation constant K_{diss} may be obtained through the equation.

Equation 4.1

$$\frac{F_o - F}{F - F_{sat}} = \left(\frac{[SWCNT]}{K_{diss}} \right)^n$$

by graphically plotting $(F_o - F)/(F - F_{sat})$ against $[SWCNT]$ in log-log scale, where F_o and F_{sat} are the fluorescence intensity of BSA solution, and that of BSA solution saturated with SWCNT, respectively (Figure 4.2(c)). The value of $[SWCNT]$ when $(F_o - F)/(F - F_{sat})$ is zero gives the value for the K_{diss} , which equals to 6.172×10^{-9} and 2.301×10^{-9} M (standard error 2.334×10^{-9} and 1.877×10^{-9} M) for pristine and carboxylated SWCNT, respectively. Higher K_{diss} value for pristine SWCNT indicates that pristine nanotubes interact less strongly with the BSA than carboxylated SWCNTs, and therefore dissociate more readily.

Figure 4.2 Fluorescence spectra from (a) pristine and (b) carboxylated SWCNT. Concentrations of pristine and carboxylated SWCNT: (1) 0.01, (2) 0.02, (3) 0.025, (4) 0.03, and (5) 0.05 mg ml⁻¹, respectively (c) Plot of $(F_0-F)/(F-F_{sat})$ against SWCNT concentration, as obtained from (a) and (b), to determine K_{diss} (--■-- carboxylated, and --●-- pristine SWCNT, respectively). CD spectra for (d) pristine and (e) carboxylated SWCNT. (f) Plot of α -helix content, as obtained from the CD spectra, against [SWCNT] (-■- carboxylated, and --●-- pristine SWCNT, respectively).

Furthermore, the change in BSA conformation is also confirmed through CD spectroscopy which measures the difference in absorbance between left and right circularly polarized light when a linearly polarized light impinges on to a molecule^[89]. Conformational features in proteins, such as α -helix, β -sheet, or random polypeptide coils typically give a certain pattern in the CD spectra in the far UV region^[87]. The α -helical content in the BSA upon interacting with pristine and carboxylated SWCNT is

asserted through interpolation ^[87] according to:

Equation 4.2

$$\alpha - helix = \frac{\theta_{measured} - \theta_{\beta}}{\theta_{pure_ \alpha} - \theta_{\beta}} \times 100\%$$

In Equation 4.2, $\theta_{measured}$, θ_{β} , and $\theta_{pure_ \alpha}$ refer to the average ellipticity angle measured from the sample, β -sheet (4000 deg cm² dmol⁻¹), and pure α -helix (33,000 deg cm² dmol⁻¹), respectively.

The change in the CD spectra (Figure 4.2 (d) and (e)) is more evident in the case of carboxylated than pristine SWCNT corroborating the fluorescence investigations. The CD spectra obtained from five different pristine SWCNTs concentrations in Figure 4.2 (a) overlaps. This is in contrast to the trend observed in Figure 4.2 (b) which displays a loss of peak intensity as the carboxylated SWCNT concentration is increased. Again, this difference indicates that BSA interacts less strongly with pristine than carboxylated SWCNT hence a less significant change in its structural conformation is expected when it is exposed to pristine SWCNT, as reflected in Figure 4.2 (d) and (e). Indeed, Figure 4.2 (f) indicates that overall, the BSA loses more of its α -helix content when it interacts with carboxylated SWCNT, as apparent from greater slope in this figure. Therefore, it can be ascertained that BSA interacts more strongly with carboxylated SWCNTs, which influences its conformation. Plainly, this difference should also have an impact on the electronic properties of the nanotube, and hence it should be reflected in the electrical kinetic reading ^[68, 72, 82].

Figure 4.3 (a) LGFET kinetic measurement, V_{ds} 10 mV, and V_G -100 mV, when the BSA interacts with pristine (black) and carboxylated SWCNT (red). (b) BSA conformation in its native conformation, and after interaction with pristine and carboxylated SWCNT. (c) The size of the binding pocket in BSA before and after interaction with carboxylated and pristine SWCNT, respectively. (d) Calculated dipole moment in (1) native BSA, (2) BSA + pristine SWCNT, and (3) BSA + carboxylated SWCNT.

Kinetic measurements in the LGFET configuration indicate that a BSA-nanotube interaction causes a drop in the current level (Figure 4.3 (a)). The drop may be attributed to positively-charged amino acid residues, such as arginine, lysine, or histidines; which present on the BSA surface. The interaction is diffusion driven, which is relatively

sluggish as indicated by the $\sim 4,000$ s time-scale. The BSA attachment to carboxylated SWCNT results in a larger current drop than pristine SWCNT which suggests more polar and charged amino acids surrounding the carboxylated SWCNT.

The semi-empirical simulation of the SWCNT-BSA interaction (Figure 4.3 (b)) reveals that pristine SWCNTs usually have only 52.8 % (S.D. 19.15%) hydrophilic and 47.2 % (S.D. 19.15%) hydrophobic residues from BSA in their surroundings. In contrast, carboxylated SWCNT typically is surrounded by 60.67 % (S.D. 10.33%) hydrophilic amino acids and 39.33 % (S.D. 10.33%) hydrophobic neighbors. Clearly, this dissimilarity gives an indication that hydrophobic interaction plays a predominant role in BSA-pristine SWCNT interaction. Whereas, in carboxylated SWCNTs, in addition to hydrophobic interactions, other factors, such as electrostatic and hydrogen bonding are expected to contribute to a stronger nanotube-BSA interaction.

This change is reflected from the size change of the binding pocket before and after interaction (Figure 4.3 (c)), where in BSA-pristine SWCNTs, the diameter of the binding pocket changes from 20.43 to 22.39 Å (S.D. 0* and 3.42 Å, respectively). On the contrary, in BSA - carboxylated SWCNT system, the diameter of the binding pocket changes more significantly from 27.6 to 33.25 Å (S.D. 0* and 11.46 Å, respectively). Furthermore, the

* The standard deviation for the initial size of the binding pocket is zero because the simulation starts from the same point. The different path taken throughout the searching routine in the simulation affects the end result, and not the beginning. This is the reason why only the size of binding pocket *after* the interaction has a standard deviation.

BSA structure was observed to influence the dipoles (Figure 4.3 (d)), with a larger dipole moment in the BSA + carboxylated SWCNT conjugate than its pristine counterpart. This change in the dipole moment and the difference in the relative amount of polar residues in proximity of the nanotubes are plausible explanations as to why the current level changes by a different degree upon exposure of the BSA towards pristine and carboxylated nanotubes (Figure 4.3 (a)).

This study has established that surface functionalization affects the interactions between proteins and SWCNTs causing changes in fluorescent signatures as well as conductance variations. The understanding on how biomolecules interact with nanotubes is thus of critical importance in SWCNT sensors that rely on electrical transduction (or optical signatures) from an immuno-complex formation for detection of cancerous antigens (e.g. prostate specific antigen), environmental toxins (e.g. pesticides), and drugs (e.g. morphine) amongst others.

4.3 Chapter Summary

In summary, fluorimetry and CD spectroscopy reveal that the BSA experiences larger conformational changes upon interacting with carboxylated SWCNTs as compared with their pristine counterparts. As suggested by semi-empirical simulations, larger conformational changes in the BSA-carboxylated SWCNT system are attributed to stronger electrostatic and hydrogen bonding interactions in addition to hydrophobic interactions observed in the pristine nanotube – BSA system. Furthermore, this different mode of interaction also influences the presence of polar and charged amino acids in

proximity of the nanotubes and results in conductance changes as detected in the liquid gated transistor configuration. This observation has significant implications on transistor-based biosensors in highlighting the need to take into account the surface functionalization states of SWCNTs.

Chapter 5 The Role of Ionic Strength on Sensing Signal of LGFET*

The electronic detection of biomolecular interaction with nanotube LGFET, as highlighted in previous chapter has a unique characteristic as compared against other approaches reported in the literatures [13, 18, 21, 66, 68, 85, 90-92]. One characteristic of this electrical detection is that it takes advantage of the charge and dipole moment presented on the surface of the nanotubes to generate the electrical signal, and as such the LGFET is capable to detect the biomolecular interaction in a real-time and label-free fashion [23, 74, 93].

Nonetheless, the operating medium of the transistor is often composed of more than merely water molecules, which adds to the complexity of the sample solution being tested. One factor that can contribute to the complication in the operation of the LGFETs is the ionic-content of the aqueous medium. In this perspective, these ions usually get attracted through electrostatic force to surfaces of opposite charge, such as those of biomolecules. Hence, upon dissolution in water, the biomolecules attract onto them a large number of ions from the surrounding aqueous medium that can lead to partial or even complete neutralization of the native charge of the biomolecules [94]. Plainly, this ion/biomolecule

* Part of the work presented in this chapter has been published: *Microfluidic-integrated nanotube and nanowire field effect transistor based biosensors* • (invited review article) • 2010 • **Micro Nanofluidic** • (accepted)

interaction which can be expected to take place in the tested sample can influence the signal level in the LGFET biosensor, and hence understanding the effect of ionic-strength to the measurement becomes a logical step in the effort to unravel the origin of signal in the LGFET and use it for biosensing applications.

Therefore, in this contribution, I would like to investigate the effect of ionic strength of the solution on LGFET sensing performance. Adsorption of a synthetic polypeptide Poly (L-Lysine) (PLL), a model molecule, to the SWCNT film here is investigated; and the ionic strength is modulated by changing the concentration as well as the type of salts in the PLL solution. The result of the experiment then is explained in the classical framework of electrical double-layer (EDL), Poisson-Boltzmann formalism, and potential distribution in aqueous media; and their relation to the transistor performance.

5.1 Experimental details

The synthetic polypeptide PLL was obtained from Sigma-Aldrich. The surfactant sodium dodecyl benzene sulfonate (SDDBS) was obtained from MD Biochemicals, Inc. The salts potassium chloride (KCl), calcium chloride (CaCl_2), and aluminum hydrochlorate (AlClH_5O_5) were obtained from Merck. The SWCNT and the PDMS were obtained from Cheaptubes, Inc and Sylgard[®], respectively.

First, a number of LGFET devices were produced using the detailed fabrication steps of the transistor as described earlier ^[72] and a series of salt solution with different ionic strength were prepared. The ionic strength in this investigation was changed by changing the cation valency, and the salt concentrations. Salt solutions (5 mM) with differing

cation valency were prepared by dissolving 30, 44, and 50 mg of KCl, CaCl₂, and AlClH₅O₅ in 40 ml of DI water. Additionally, a logarithmic dilution series of KCl solution was also prepared to investigate the effect of salt concentration: 1, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, and 10⁻⁶ M. A series of PLL solution in these salts (5×10⁻³ w/v %) were prepared as test solution, while blank salt solutions were used as control.

During the measurement, the V_G and V_{DS} were fixed at -500 and 10 mV, respectively and the I_{DS} was measured and plotted as a function of time in kinetic mode. Baseline measurements were performed with every salt solution by measuring the kinetic behavior of the LGFET in blank salt solution in absence of PLL. A sufficient amount of time is allowed to stabilize the device kinetic response before and after the PLL solution is introduced.

Additionally, a series of electrostatic potential calculations was also performed with *Accelrys DS Visualizer*. An efficient algorithm in the software solved the coupled wave function equation to give the electrostatic potential map of the molecule projected on to the molecule's surface. This electrostatic potential distribution represents the charge distribution on the surface of the protein. The resulting electrostatic potential map is useful to give hindsight about how the charge distribution on the protein surface influences the signal level in the LGFET.

5.2 Results and Discussion

Most biomolecules have a large size, complex and constituted from many molecular units which can confer a hydrophilic and/or hydrophobic character. However, the hydrophobic

parts of the biomolecules are usually buried inside and the surface of the biomolecules is decorated primarily by the hydrophilic constituents. This instantaneous self-arrangement takes place because the environment in which these biomolecules reside is primarily that of aqueous media.

The hydrophilicity of these units typically arises because they either have a net charge, or a dipole moment. The electric field that comes from these constituents, consequently, attracts opposite ions from the aqueous media, which leads to formation of EDL and results in partial/complete charge neutralization. Therefore, understanding the influence of EDL on the LGFET behavior is important for fundamental and practical reasons. The understanding is fundamentally important, because it allows us to understand the complexity of the biomolecular interaction, and the environment in which this takes place. Theoretically, at least, an understanding about the effect of EDL allows us to predict the logical spatial distance from the nanotube surface in which a biomolecular interaction would give a signal at a particular ionic strength condition.

This last point is also important from practical perspective, because this means that the signal may come only from a local interaction, instead of the entire molecules, depending on the ionic strength of the background solvent as well as the size of the biomolecule in question. Additionally, understanding the effect of EDL is also of practical importance, because it allows to identify a window of ionic-strength at which the LGFET can operate reliably as a biosensor. This point becomes crucial because of the fact that most body fluids typically have a relatively high salt concentration. Hence, understanding how the LGFET biosensor behaves under such condition becomes very important, so that the

the origin of the signal can be evaluated: local interaction, or the entire molecule interaction.

PLL is a synthetic polypeptide which is widely used in biochemical experiments, especially for passivation of glass surfaces. Structurally, the PLL molecules are quite simple in that it consists of only Lysine amino acid residues, and its conformation consists entirely of α -helix (Figure 5.1 (a)). Furthermore, the Lysine residues gives the PLL net positive charge, which is distributed evenly throughout the molecules, as shown in Figure 5.1 (b).

Figure 5.1 (a) PLL is a synthetic polypeptide which consists entirely from Lysine amino acid residues, and whose structure is mainly α -helix. Here shown in (a) is the wireframe and ribbon representation of the polypeptide. The α -helix structure is clearly shown in the ribbon representation. (b) The Lysine residues impart net positive charge on the polypeptide surface. This positive charge is distributed evenly throughout the PLL molecules, as shown in this figure.

The electrical behavior of the LGFET can be described mathematically by the classical transistor-action equation:

Equation 5.1

$$I_{DS} = \frac{\mu}{2} C_{ins} \frac{W}{L} (V_G - V_T)^2$$

In which μ , C_{ins} , W , L , and V_T are to denote the charge carrier mobility, capacitance per unit area, width, and length of the transistor electrical channel, and threshold potential, respectively. Equation 5.1 implies that the I_{DS} - V_G curve of LGFET can be modulated by changing the V_G as well as the V_T . While V_G can be modulated by changing the applied bias through an external voltage source, changes in V_T typically is due to interaction between the nanotubes and the biomolecules. Specifically, the V_T is typically governed by equation ^[95]:

Equation 5.2

$$V_T = \Delta\phi_{ms} - \frac{\sum Q_i}{C_{ins}}$$

In which $\Delta\phi_{ms}$ and Q_i are to denote the difference between work function of the metal contact and the SWCNT, and the charge that interacts with the nanotube, respectively. Hence, modulation of Q_i would lead to changes in the I_{DS} level, as reflected in the shift of the I_{DS} - V_G and kinetic data- a mechanism which is also known as electrostatic gating.

Physically, the Q_i in Equation 5.2 influence the transistor behavior through disruption of

the potential distribution surrounding the nanotube. In the case of PLL, the positive charge from the molecules shifts the I_{DS} - V_G curve toward negative bias direction (Figure 5.2 (a)). Or, kinetically, this shift can be observed as a decrease in the I_{DS} as a function of time (Figure 5.2 (b)).

Figure 5.2 (a) I_{DS} - V_G curve from the LGFET, and (b) its corresponding kinetic curve. The I_{DS} - V_G is shifted toward negative bias direction upon adsorption of PLL onto the nanotubes. This shift is caused by disruption of potential landscape surrounding the SWCNT, and changes the V_T value to become more negative. The negative shift in the I_{DS} - V_G is reflected as a decrease in I_{DS} in kinetic measurement (b) ^[72].

In this framework, the ionic-strength of the solution influences the signal level in the LGFET through two possible scenarios: (1) the positive charge originated from the PLL is screened either partially or completely by attraction of counter-ions from the solution unto the molecules, and/or (2) the counter-ions screen the innate negative charge from the nanotube surface, and hence possibly leads to less-effective interaction between the two molecules. As noted, in both of these possible scenarios the signal level in the LGFET

can be expected to be influenced by the ionic-strength of the aqueous medium.

Figure 5.3 shows the effect of ionic-strength on the signal level in the LGFET biosensor, which in this case is modulated by changing the valency of the ions (Figure 5.3 (a)), and/or the concentration of the salts (Figure 5.3 (b)). As noted, the signal level in the LGFET decreases as the ionic-strength of the solution increases, indicating that high ionic-strength solution is more effective in screening the charge from the PLL.

Figure 5.3 Kinetic response of the LGFET upon exposure toward PLL in different ionic-strength. Modulation of the ionic-strength is obtained by changing the (a) valency of the ions, or (b) the concentration of the salt. In both cases, the signal level of the LGFET decreases as the ionic-strength of the solution increases. This diminution of the signal level is because the ions screen-off the charge from the PLL. The effectiveness of this screening depends on the ionic-strength of the solution.

Classically, the potential distribution around charged molecules in aqueous media is governed by Poisson-Boltzmann equation:

Equation 5.3

$$\nabla^2 \psi = -\frac{1}{\varepsilon_r \varepsilon_o} \sum_i^n z_i F c_o \exp\left(-\frac{z_i F \psi(x)}{RT}\right)$$

In which ψ , ε_r , ε_o , z_i , c_o , F , R , and T are to denote the potential, dielectric constant, permittivity of vacuum ($8.85 \times 10^{-12} \text{ F m}^{-1}$), valency of the ion, ion-concentration, Faraday constant ($96,500 \text{ C mol}^{-1}$), gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), and absolute temperature, respectively. In general, the Poisson-Boltzmann equation has to be solved numerically, because of its partial-differential and non-linear nature. However, in dilute ionic solution, Debye-Huckel approximation allows solving Equation 5.3 analytically by approximating the exponential term with first order Taylor expansion:

Equation 5.4

$$\psi = \psi_s \exp\left(-\frac{x}{\kappa^{-1}}\right)$$

This equation reveals that the molecular surface charge induces a potential which decays exponentially in the bulk solution. This decaying trend is a direct contribution from the screening from the ions in the EDL. The rate of this decay is governed by the famous Debye length κ^{-1} :

Equation 5.5

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_r \varepsilon_o kT}{2 \sum_i (z_i e)^2 c_o}}$$

Or, by employing definition of ionic-strength

Equation 5.6

$$I_{strength} = \frac{1}{2} \sum_i (z_i e)^2 c_o$$

The Equation 5.5 can be re-casted into

Equation 5.7

$$\kappa^{-1} = \sqrt{\frac{\epsilon_r \epsilon_o kT}{4I_{strength}}}$$

Taking conservative ϵ_r value of 78 for water, and assuming of surface potential of 10 μV , the potential distribution around the PLL can be calculated according to Equation 5.4 for different ionic-strength (Figure 5.4). Hence, Equation 5.7 and Equation 5.4 show that the screening of the potential from the PLL is more effective when the ionic-strength of the solution is increased. This theoretical result is clearly observed in the experimental data Figure 5.3 (a) and (b), whereby an increase of ion valency and concentration leads to degradation of the LGFET signal.

Figure 5.4 Potential distribution around the PLL at (a) different ion valency, and (b) ion concentrations. The decaying rate increases when the ionic strength of the solution is increased. This screening, in turns, influences the transistor behavior, as revealed in Figure 5.3.

Alternatively, the κ^{-1} can also be thought as the furthest interaction distances which still capable to give a signal in the LGFET measurements. From Equation 5.5, the κ^{-1} at differing conditions employed in this experiment can be calculated (Table 5.1). As noted, the κ^{-1} changes more significantly from 2.2 to .1.2 nm when the ion valency is changed from 1 to 3. This is in contrast to the rather sluggish change from 21.5 to 0.2 nm when the concentration of the ions is changed from 10^{-4} to 1 M. This trend is rather expected, because the κ^{-1} is inversely proportional to z_i , while it is only inversely proportional to the square root of c_o ; and hence making κ^{-1} more sensitive to changes in the z_i than c_o .

Table 5.1 Debye length at different ionic-strength, as manifested by different ion valency, and ion concentration in the salt solution

	z_i (ion concentration 10 mM)			Ion concentration (M) ($z_i = 1$)		
	1	2	3	10 ⁻⁴	10 ⁻²	1
κ^{-1} (nanometer)	2.2	1.2	1.2	21.5	2.2	0.2

The calculation result shown in Table 5.1 also gives information regarding the likely source of the signal upon interaction of biomolecules with the c-SWCNT. The relatively small κ^{-1} values indicate that at moderate ionic strength solutions, such as that of body fluids, most of the signal in the LGFET is contributed from local interaction between the biomolecules and the c-SWCNT.

For example, the common composition of a 50 mM phosphate buffer solution is: 50 mM Na_2HPO_4 , and 50 mM of NaH_2PO_4 . From this information, the κ^{-1} can be calculated in the proximity of 0.5 nm. Note that this κ^{-1} value is much smaller than the typical size of a protein which in the range of 9 nm. Therefore, when a protein is attached on to a c-SWCNT, only those amino acid residues closest to the c-SWCNT can actually influence the conductivity of the nanotube. This explains why in previous chapter, the LGFET shows a decrease in the I_{DS} , which marks presence of positive charge; upon bovine serum albumin (BSA) adsorption, even though the protein has a net negative charge at pH 7.4 (Figure 4.3 (a)).

Figure 5.5 (a) Kinetic response from the LGFET upon BSA adsorption (phosphate buffer, pH 7.4, 50 mM) shows a decrease in I_{DS} . (b) Binding pocket of carboxylated SWCNT upon interaction with BSA, and (c) charge distribution in the BSA protein. Note that even though the BSA overall has negative charge, the nanotube is surrounded by amino acid residues which are mostly positively charged and polar. This data indicates that the signal in the LGFET comes predominantly from local interaction between the nanotube and the BSA.

Figure 5.5 (a) show the kinetic response from the LGFET upon BSA exposure in phosphate buffer (pH 7.4, 50 mM). The figure shows that the I_{DS} decrease upon BSA adsorption, which indicates presence of positive charges; even though the protein has an overall negative charge at this pH value. Figure 5.5 (b) and (c) show the binding pocket of carboxylated upon interaction with the BSA, as well as the corresponding charge distribution in the BSA surface, respectively. The later figure displays that although the BSA has an overall negative charge at pH 7.4, the carboxylated SWCNT tends to interact with a binding pocket rich in polar amino acids. The comparatively small κ^{-1} values indicate that most of the I_{DS} modulation can only come from local interaction between the c-SWCNT and these neighboring amino acids, and hence explains the reason behind the

I_{DS} drop observed in Figure 5.5 (a).

5.3 Chapter summary

In this chapter, the effect of ionic strength on the sensing signal level of the LGFET has been explained. The investigation is important because most biomolecules interact with the device due to their innate electric field. Nevertheless, the presence of these innate electric fields also attracts counter-ions from the surrounding solution, and lead to formation of the EDL. Naturally, this EDL formation leads to screening of the charge from the biomolecules surface, influencing their interaction with the c-SWCNT and hence, the device signal level. The extent of this screening is dictated by the ionic-strength of the solution. Particularly, increasing the ionic-strength through both increasing the valency or the total concentration of the ions; leads to more effective screening and diminishes the signal level in the device.

The extent of this screening is stated in terms of Debye length κ^{-1} . In relatively-high ionic-strength solutions, the value of κ^{-1} is only few nanometers and explains why the signal level diminishes as the ionic-strength is increased. Alternatively, the low κ^{-1} values also inform that at high ionic-strength, the I_{DS} modulation is caused predominantly by local interaction between the protein and the c-SWCNT. This short local interaction explains why the LGFET displays a decrease in I_{DS} , which indicates presence of positive charge; upon BSA adsorption, even though the protein has a net negative charge at pH 7.4.

Chapter 6 Femto Molar Detection of 2, 4-dichlorophenoxyacetic acid Herbicides via Competitive Immunoassays in a Microfluidic Based Carbon Nanotube Liquid Gated Transistor*

Size similarity of nanotubes and most biomolecules allows for the possibility of a mutual interaction at a molecular level ^[96] that induces specific changes, in both the biomolecules and the nanotubes ^[97], thus enabling development of novel biosensing strategies ^[73, 98].

For instance, single-walled carbon nanotubes (SWCNTs) have been employed in detection of deoxyribonucleic acid (DNA) based on both electrochemical and transistor based configuration and detection limits of the order of parts per trillion ^[5, 20] have been reported. Detection of glucose down to concentration of 18 $\mu\text{g ml}^{-1}$ has also been achieved. Sensors for real-time detection of analytes in liquid media have been reported based on sensors integrated with microfluidic channels ^[99], however most devices still rely on silicon materials and microlithography techniques for the sensor fabrication and high temperature processes for SWCNT growth.

* The work presented in this chapter has been published: *Femtomolar detection of 2, 4-dichlorophenoxy acetic acid herbicides via competitive immunoassays using microfluidic based carbon nanotube liquid gated transistor* • 2010 • **Lab on a Chip** • Vol 10, p 634 – 638

Monitoring of environmental pollutants has become increasingly important in the recent years, due to growing public awareness of environmental impact of agro-chemical advancements and environmental legislation pursued by governments in response to the potential health hazards posed by these pollutants. The wide use of herbicides, fungicides, and pesticides has contributed to the contamination of not only soil, but also ground and surface water as well as the oceans. One such herbicide, 2, 4-dichlorophenoxyacetic acid (2, 4-D), is widely used for the control of broadleaf weeds in agriculture, and forestry; and has been shown to be very harmful to aquatic life ^[100]. Conventionally, herbicide analysis is carried out by high-performance liquid chromatography (HPLC). However, this technique is expensive, consumes large amounts of solvent, and the instrumentation required is bulky. Hence, an in-situ, real-time, low-cost, field-deployable sample prescreening method is a much sought-after solution.

In this chapter, I will focus on detection of 2, 4-D in soil extract with an all-plastic SWCNT liquid-gated field effect transistor (LGFET) ^[72] using a competitive immunoreaction for the selective detection of 2, 4-D. The proposed immunosensor is extremely sensitive for the detection of 2, 4-D achieving a limit of detection of 500 fM levels in soil extracts.

6.1 Experimental Details

Technical grade 2, 4-dichlorophenoxy acetic acid (2, 4-D), bovine serum albumin (BSA), N-hydroxysuccinimide ester (NHS), N',N'' dicyclohexylcarbodiimide (DCC), Dimethyl formamide (DMF), keyhole limpet hemocyanine (KLH), Freund's complete adjuvant

(FCA), Freund's incomplete adjuvant (FIA), goat anti-rabbit IgG-HRP conjugate, trifluoroacetic acid (TFA) were purchased from *Sigma-Aldrich*. 3, 3', 5, 5'-tetramethylbenzidine (TMB) was purchased from *Bangalore Genei*. Protein A sepharose and sepharose 4 B were procured from *Pharmacia*. ELISA plates (*Nunc F96 MicroWell*) were obtained from *Maxisorp*. Disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from *Merck*. Tween-20 blocking agent and sodium-dodecyl benzene sulfonate (SDDBS) were purchased from *Sigma-Aldrich*. Poly (dimethyl siloxane) (PDMS) (Sylgard 134) was purchased from *DuPont*, and carboxylated SWCNTs (2.5 atomic %) were purchased from *Cheaptubes, Inc.*

The Phosphate Buffer (PB) solution was prepared by mixing the Na_2HPO_4 and NaH_2PO_4 solution until pH 7.4 (50 mM) was attained. The preparation of antibodies against 2, 4-D (anti-(2, 4-D)) has been reported in detail in a previous study ^[101] and schematically shown in Figure 6.1 (a). Briefly, the hapten was first prepared by mixing the BSA and the 2, 4-D in the presence of DCC + NHS ^[102] carbodiimide linker chemistry for covalent binding. Additionally, the 2, 4-D was also conjugated with KLH protein for coating of ELISA plates with similar linking strategy. All of the conjugates were then purified with a P10 gel filtration column (*Pharmacia*) and the fractions with maximum protein concentration were collected and stored in aliquots at -20°C . Subsequently, six- to eight-weeks old New Zealand white rabbits were immunized subcutaneously with 300-400 μg of the 2, 4-D/BSA emulsified in FCA. Succeeding booster doses were given with FIA at the interval of 21 days. Afterward, the blood was collected and the serum was precipitated. The immunoglobulin (IgG) fractions were purified from the sera using

protein-A sepharose column and the bound antibodies were eluted with 0.1M glycine-HCl buffer (pH 2.5). The eluted IgG fractions were pooled and dialyzed against PBS pH 7.4 at 4 °C, and stored in aliquots at –20 °C.

Figure 6.1 Protocol for (a) preparation of 2, 4-D/BSA conjugate and anti-(2, 4-D), and (b) preparation of soil extract.

Soil samples were obtained from a plant nursery, and to prepare the soil extract, one gram of soil was dispersed in a 50 ml of DI water (Millipore). The suspension was then sonicated for two hours for homogenization. Afterward, the suspension was centrifuged (3000 rpm) for 30 minutes to separate large particulates from the soil extract solution (Figure 6.1 (b)).

Transistor measurements were performed by applying a gate bias (V_G) with a function generator (*Thurlby Thandar Instrument, TTi TG1304*), connected to a Ag/AgCl reference

electrode (*World Precision Instruments*). The V_G applied was also monitored with a digital oscilloscope (*Agilent DSO3062A*). A voltage source/ pico-ampere meter module (*Keithley 6487*) was used to supply the source-drain bias (V_{DS}) and measure the source-drain current (I_{DS}) at the same time. All the electrical instruments were synchronized by a personal computer through a general purpose interfacing bus (GPIB) card (*National Instrument*) and a programming code written in *LabVIEW 7.1*.

Direct detection of 2, 4-D and anti-(2, 4-D) was performed through I_{DS} - V_G measurement. For the former, the anti-(2, 4-D) antibodies (5 μ M) were first attached on to the nanotube surface to act as the probe molecule. Then, free un-conjugated 2, 4-D (5 μ M) was introduced into the microfluidic channel. Similarly, in the case of direct detection of anti-(2, 4-D), the nanotube surface was first incubated with 2, 4-D/BSA conjugate (5 μ M) and subsequently, the device was exposed to the antibodies solution (5 μ M). The immuno-complex formation in both assays was detected by monitoring the I_{DS} - V_G curve before and after the immuno-reaction.

Additionally, kinetic measurements were also performed in triplicate for: (1) detection of anti-(2, 4-D) with direct immunoassay in PB buffer (pH 7.4, 7.3 mS cm^{-1}), (2) detection of the free 2, 4-D by competitive immunoassay in PB buffer (pH 7.4, 7.3 mS cm^{-1}), and (3) detection of the free 2, 4-D by competitive immunoreaction in soil extract (pH 7, 12 μ S cm^{-1}). In all three cases, the 2, 4-D/BSA conjugate was used as the probe molecule to capture the anti-(2, 4-D). Logarithmic serial dilutions of 2, 4-D or anti-(2, 4-D) from 5 fM up to 500 μ M were prepared to determine the detection limit of the LGFET at different conditions. In the direct immunoassay protocol, anti-(2, 4-D) at different

concentrations was directly applied to the LGFET, which had been initially functionalized with the 2, 4-D/BSA conjugate. In the case of the competitive immunoassay protocol, a fixed concentration of anti-(2, 4-D) (50 μ M) was first incubated for approximately two hours at room temperature with free 2, 4-D solution in PB buffer or soil extract at different concentrations, and then injected to the device for the sensitivity study. Selectivity of the device was investigated by exposing a non-pairing protein, such as native BSA to the 2, 4-D/BSA conjugate while monitoring the I_{DS} . All measurements were repeated at least three times to determine the repeatability and the standard deviation of the data.

6.2 Results and Discussion

Figure 6.2 Schematic of direct assay detection of (a) free 2, 4-D and (b) anti-(2, 4-D), and the corresponding I_{DS} - V_G response ((c) and (d)), respectively. In the former, the 2, 4-D and anti-(2, 4-D)

are the target and probe molecule, respectively. Detection of anti-(2, 4-D) is the reverse situation of direct 2, 4-D detection, whereby here the 2, 4-D/BSA conjugate and the anti-(2, 4-D) are the probe and target molecules, respectively.

SWCNTs comprise single layers of graphene sheets wrapped into a nano-sized tubular structure, and consequently charge-carriers are confined purely to the surface of the nanotubes. The conductivity of nanotubes is thus very sensitive to slightest electrostatic disturbances in its surroundings. Biomolecules/SWCNT interactions lead to such a electrostatic potential disturbance and modulates the nanotube conductance, hence providing a facile transduction mechanism that may be discerned with a LGFET.

In the direct immunoassay (Figure 6.2 (a)), the probe molecule anti-(2, 4-D) is attached on to the nanotube surface, while the free 2, 4-D in solution functions as the target molecule. The baseline in transfer characteristic (Figure 6.2 (c)) is recorded after the anti-(2, 4-D) has been attached on to the nanotube, and the unbound SWCNT surface has been passivated with the tween-20 blocking agent. It may be noted that the I_{DS} - V_G curves before and after the 2, 4-D exposure completely overlap indicating that the device is not able to detect the immuno-complex formation between the free 2, 4-D and anti-(2, 4-D).

In the direct immunoassay detection of the anti-(2, 4-D) (Figure 6.2 (b)), the 2, 4-D/BSA conjugate functions as the probe, while the anti-(2, 4-D) is the target molecule. In contrast to the previous observation (Figure 6.2 (c)), the I_{DS} - V_G characteristic now displays a considerable shift toward negative V_G direction (shown in Figure 6.2 (d)).

The differing response levels may be attributed to the large size difference between the free 2, 4-D ($221.04 \text{ g mol}^{-1}$) and the anti-(2, 4-D) ($1.5 \times 10^4 \text{ g mol}^{-1}$). When used as the

probe molecule, anti-(2, 4-D) introduces a large background charge, thus dwarfing the small potential disturbance that the free 2, 4-D inflicts upon the immuno-complex formation.

In contrast, binding of anti-(2, 4-D) toward the 2, 4-D/BSA conjugate introduces significant potential disturbance surrounding the nanotubes, hence leading to a shift of the I_{DS} - V_G curve toward negative V_G values (Figure 6.2 (d)), indicating that the modulation comes primarily from electrostatic gating. This negative shift normally is associated with enrichment of positive charge that results from the immuno-complex formation.

In general, possible sensing mechanisms in LGFETs include electrostatic gating, Schottky barrier, capacitance, and mobility modulation ^[23], each of which records a characteristic change in the I_{DS} - V_G curve. Electrostatic gating is associated with a shift in I_{DS} - V_G curve (positive or negative), depending on the charge of the biomolecules binding to the nanotubes. Modulation of the Schottky barrier is characterized by a decrease in I_{DS} at negative V_G bias, and increase in I_{DS} at positive V_G bias. A change in the capacitance is typified by modulation in the saturation current, whereas mobility changes caused by scattering or other effects of biomolecule/SWCNT interaction show a decrease in slope under both positive and negative V_G bias.

It can be inferred from the I_{DS} - V_G shift in Figure 6.2 (d) that the immuno-complex primarily influences the nanotube through electrostatic gating modulation. Furthermore, the slope of I_{DS} - V_G curve does not seem to be strongly affected from the immuno-complex formation, indicating that the charge-carrier mobility in the nanotube is

unchanged following the immuno-reaction.

Figure 6.3 (a) Kinetic data recorded during the immuno complex reaction. Concentrations of anti-(2, 4-D): (1) 5 fM, (2) 50 fM, (3) 5 pM, (4) 50 pM, (5) 5 nM, (6) 50 nM, (7) 5 μ M, (8) 50 μ M, and (9) 500 μ M; to test the sensitivity of the device. (b) Calibration curve resultant from the immuno-reaction. (c) Selectivity of the LGFET was verified by exposing the device toward native BSA (500 μ M) to the 2, 4-D/BSA conjugate (500 μ M).

The immuno-complex formation in real-time was also probed by using the LGFET in kinetic mode. In the first set of kinetic experiments, I have tested the device for detection of anti-(2, 4-D) in PB buffer (pH 7.4, 7.3 mS cm^{-1}) in direct immunoassay format. In this

case, the 2, 4-D/BSA conjugate was first covalently immobilized on to the SWCNT with the aid of standard carbodiimide linker chemistry. The LGFET was then employed to capture free anti-(2, 4-D) in the solution, while measuring the I_{DS} in real-time and keeping the V_G values constant. Figure 6.3 (a) reveals the kinetic response from the device upon exposure toward anti-(2, 4-D) solution at increasing concentrations. The I_{DS} here has been normalized to the baseline value (I_o) to remove variability that comes from different number of nanotubes on the device. The decrease in I_{DS} indicates that the immuno-complex formation results in fortification of positive charge surrounding the nanotube, supporting the data in Figure 6.2 (d). This positive charge can be attributed to basic amino acids residues, which usually populates the epitopic binding pocket of the antibodies. ^[103, 104]

Expectedly, this drop in I_{DS} becomes larger as the anti-(2, 4-D) concentration in the solution is increased (Figure 6.3 (a)). The positive correlation between I_{DS} drop and anti-(2, 4-D) concentration is typical in direct immunoassay tests, because the signal level is directly proportional to the concentration of the antibodies.

The general trend of the I_{DS} drop at different anti-(2, 4-D) concentrations is noticeable in the calibration curve (Figure 6.3 (b)) which represents three kinetic measurement replicates. The calibration data was fitted by the regression equation ^[105]

Equation 6.1

$$\frac{I_{DS}}{I_o} = a - b \log([anti - (2,4 - D)] + c)$$

yielding a correlation factor $R^2 = 0.957$ indicating a good fit between the regression and the underlying data points. The sensitivity of the LGFET in Equation 6.1 is associated with its slope, which equals to $\sim 0.21 \text{ \% decade}^{-1}$. The standard error of the regression is taken as the square root of residual variance from the test of analysis of variance (ANOVA) ^[106] in the regression routine. The detection limit is then determined from the 3σ criterion ^[72, 106], yielding value approximately 50 pM for the case of detection of anti-(2, 4-D) in PB buffer.

The selectivity of the device was also investigated by exposing the LGFET toward a non-pairing protein, such as native BSA. Figure 6.3 (c) shows that the I_{DS} drop only slightly upon exposure to the native BSA, also confirming the effectiveness of tween-20 as the blocking agent which prevents attachment of excess biomolecules directly to CNTs and potentially interfering with the sensing response.

Figure 6.4 (a) Schematic of competitive assay protocol. In this protocol, the free 2, 4-D is detected indirectly. (b) Kinetic response from the LGFET upon exposure of free 2, 4-D solution in PB buffer (pH 7.4, 7.3 mS cm^{-1}) of differing concentrations: (1) $50 \text{ }\mu\text{M}$, (2) 500 nM , (3) 50 nM , (4) 5 nM , (5) 500 pM , (6) 50 pM , (7) 5 pM , (8) 500 fM , (9) 50 fM , and (10) 5 fM . (c) Calibration curve resultant from the immuno-reaction. Inverse relationship between the signal and the free 2, 4-D concentration is clearly observed in this figure.

As discussed earlier (Figure 6.2 (c)), the detection of free 2, 4-D using a direct immunoassay protocol is not possible due to its small size as compared to the anti-(2, 4-D). An alternative to detect the free 2, 4-D in solutions is through the competitive immunoassay protocol. For this set of experiments, a fixed concentration of anti-(2, 4-D) ($50 \text{ }\mu\text{M}$) was mixed with a series of free 2, 4-D solutions differing in concentration. Upon

injection into the LGFET, the free 2, 4-D molecule competes with the 2, 4-D/BSA conjugate to bind with the anti-(2, 4-D) in solution (Figure 6.4 (a)). Therefore, the larger the concentrations of free 2, 4-D in the aqueous media, the less is the number of anti-(2, 4-D) that binds with the 2, 4-D/BSA conjugate. Hence, the competitive assay protocol yields a calibration plot that is inversely proportional to the concentration of free 2, 4-D. The kinetic response (Figure 6.4(b)), however, is similar to direct immunoassay protocol (Figure 6.3(b)), because in both cases, the device detects binding of anti-(2, 4-D) to the 2,4-D/BSA conjugate.

The inverse relationship between current and concentration of 2, 4-D is also observed in the calibration curve (Figure 6.4 (c)) which is derived from three kinetic measurement replicates. As described earlier (Equation 6.1), the R^2 0.995 indicates excellent regression fit with sensitivity of $\sim 0.33\% \text{ decade}^{-1}$, and a 2,4-D detection limit of $\sim 50 \text{ pM}$ - far lower than the World Health Organization (WHO) maximum residual level of 2, 4-D concentration in drinking water^[107] at 500 nM levels.

In the third set of experiments, the competitive immunoassay is employed to detect the free 2, 4-D in the real sample of soil extract with the LGFET. The pH and the conductivity of the soil extract are found to be about 7, and $12 \mu\text{S cm}^{-1}$, respectively. This indicates that even though the soil extract has a pH similar to that of lab condition (pH 7.4, and conductivity 7.3 mS cm^{-1}), the ionic-strength of the extract is much lower than those tested in the lab.

Figure 6.5 (a) Kinetic measurement upon exposure to the free 2, 4-D in soil extract in competitive immunoassay format. The I_{DS} decreases upon exposure toward the free 2, 4-D with different concentrations: (1) 5 μ M, (2) 500 nM, (3) 50 nM, (4) 5 nM, (5) 500 pM, (6) 50 pM, (7) 5 pM, (8) 500 fM, (9) 50 fM, and (10) 5 fM. (b) The calibration curve shows inverse relationship between the level of signal and the free 2, 4-D concentration, typical for competitive immunoassay. The calibration curve is obtained from three replicates of kinetic measurements.

It may be noted from the kinetic response and calibration plots (Figure 6.5 (a,b)) that the change in I_{DS} is almost similar to those observed in Figure 6.4 (b) and (c), except that it is somewhat larger in the case of soil extract. This slight amplification is attributable to the lower ionic-strength ($12 \mu\text{S cm}^{-1}$) of the soil extract as compared to PB buffer solution (7.3 mS cm^{-1}). In aqueous solutions, the polar and/or charged surface of biomolecules attracts counter-ions from their surroundings. These counter-ions screen the stray potential which originates from the biomolecules and is required to disturb the conductance of the SWCNTs. In higher ionic-strength solutions, this screening process is

more effective than in lower-ionic strength and hence, the signal in LGFETs is inversely proportional to the ionic-strength of the solution ^[108], and probably is the reason why the change in I_{DS} is slightly larger in the case of soil extract than in PB buffer.

The correlation factor R^2 of 0.989 as extracted from Equation 6.1 and the sensitivity of $0.94\% \text{ decade}^{-1}$ is higher than the previous two cases and the limit of detection is estimated at 500 fM. The improvement in the sensitivity and limit of detection over the case of 2, 4-D detection in PB buffer is a direct consequence of the larger I_{DS} drop observed in the soil extract.

6.3 Chapter Summary

In this chapter, direct, indirect and competitive immunoassays for the detection of a commonly used herbicide 2, 4-D has been applied. The detection as extracted from the drain current response of the LGFETs yielded a real-time, label-free detection of free 2, 4-D molecules with extremely low detection limits of ~500 fM (0.5 part-per-trillion) and 50 pM (50 part-per-trillion) in soil extract and buffer solution, respectively. These detection limits are much lower than the maximum residual limit of 500 nM (500 part-per-billion) for the 2, 4-D established by the WHO in drinking water.

The instrumentation and technology developed is simple and low cost, hence, has the potential for in situ analysis of environmental samples including detection of water borne toxins or in the medical field to replace some of the immunoassays based diagnostic methodologies currently performed.

Chapter 7 Signal amplification in carbon nanotubes based liquid-gated transistor biosensing device via pulsating gate bias*

As discussed in earlier chapter, the ability to operate the nanotube transistors in kinetic mode means that the biomolecular interactions may now be probed in real-time in their most native environment, aqueous medium. Nonetheless, the ability of the transistor to operate in liquid environments also comes with a limitation in that traditionally, the operating applied gate bias (V_G) can not exceed ± 1 V because electrolysis of water takes place with disrupting consequences of the LGFET functioning. This limitation hence constrains the device operating voltages and restricts the drain current and signal-to-noise levels employed for the sensing transduction. In the previous chapter, a LGFET platform have been introduced which is based solely on transfer-printed SWCNTs on a Poly-(dimethyl siloxane) (PDMS) carrier capable of detecting protein adsorption with detection limits of ~ 1 pM^[72] within a voltage range of ± 1 V. In this chapter, a pulsed-gating approach that extends the LGFET operating window beyond the electrolysis boundary is demonstrated. This pulse-gating approach is also shown to be an effective

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methodology to amplify the sensing signal and now enables the detection of the model Poly-L-Lysine (PLL) protein down to atto-molar concentrations.

7.1 Experimental details

The SWCNTs were purchased from *Carbon Solutions, Inc.* The carboxylation of the nanotubes was then performed through acid treatment as reported in the literature ^[86]. Sodium-dodecyl benzene sulfonate (SDDBS) and PLL were purchased from *Sigma-Aldrich*, and PDMS (Sylgard 134) was procured from *DuPont*. The LGFET device was prepared as detailed in Chapter 3.

For the purpose of sensitivity study, a logarithmic serial dilution of PLL was prepared in de-ionized water (*MilliQ*) at increasing concentrations: (1) 0.2 aM, (2) 2 aM, (3) 20 aM, (4) 200 aM, (5) 2 fM, (6) 20 fM, (7) 200 fM, (8) 2 pM, (9) 20 pM, (10) 200 pM, (11) 2 nM, (12) 20 nM, (13) 200 nM; and the incubation time was estimated by calculating the diffusion time in the micro-channel according to the Fick's second law of diffusion with diffusivity as reported in the literature ^[109].

Additionally, simulation on how the biomolecular interaction influences the transistor characteristic was performed in *MATLAB 6.5* according to Landauer-Buttiker formalism as reported in the literature ^[23, 50]. Briefly, in this equation, the electrical current that passes through the nanotube is treated as modulation of tunneling of charge carriers through two Schottky barriers at the source and drain junctions due to the act of the gate bias, as dictated by:

Equation 7.1

$$I_{DS}(V_G) = \frac{4q}{h} \sum_i \int_{E_1}^{E_2} T_n(E, V_G) (f_S(E) - f_D(E, V_{DS})) dE$$

In this formalism, the summation is performed throughout the conducting channels in the nanotubes, and the integration was carried out from the smallest energy level in the conduction band to the first Brillouin-zone of the energy-momentum diagram of the nanotube. Following the approach given in the literature ^[23], the effect of non-idealities which contribute to diffusive transport in the nanotube is modeled by cascading the conductance as found from the Landauer-Buttiker formalism in series with the classical Drude conductance. Subsequently, the behavior of the I_{DS} - V_G curves responding toward the biomolecular event at both $|V_G| < 1$ and $|V_G| > 1$ volt were simulated, and compared with that of experimental result.

The ability of the LGFET for identifying bioanalytes in real-life sample proxy was assessed by employing the device for detection of p53 (*BD Bioscience*) head-and-neck cancer biomarker in 100x diluted artificial saliva (*OralBalance Biotène*) in phosphate buffer (pH 7.4, 50 mM). Considerable size difference between the p53 (48 kD) and the corresponding p53 antibody (anti-p53) (~ 150 kD) ^[110] necessitates the engagement of competitive immunoassay protocol for the indirect detection of the p53 bioanalytes. In this procedure, the p53 (1 μ M) was first conjugated with the SWCNT to function as a probe molecule, followed by incubation in tween-20 (*Sigma-Aldrich*) for two hours to prevent non-specific binding from taking place. Subsequently, a solution of anti-p53 (1 μ M) and a logarithmic serial dilution of p53 bioanalytes in artificial saliva with

increasing concentration: (1) 0.1 fM, (2) 10 fM, (3) 1 pM, (4) 100 pM, (5) 10 nM, and (6) 1 μ M; were prepared.

The prepared p53 and anti-p53 solution were then pre-mixed and let stand for at least three hours before introducing to the microfluidic channel for the electrical measurement. The selectivity of the LGFET biosensor was examined by exposing the device toward anti-p53 (1 μ M) and non-pairing protein, such as free BSA (1 μ M) while performing the LGFET measurement in kinetic mode.

7.2 Results and Discussion

Figure 7.1 (a) A schematic of all-plastic SWCNT LGFET, (b) Pulsating V_G applied to the reference electrode to avoid electrolysis at $|V_G| \geq 1$ volt. (c) I_{DS} - V_G obtained with $-2 \geq V_G \geq -0.5$ volt operating

window with pulsed-gate (continuous black line) and DC sweep (dashed red line). (d) The effect of pulsed-gate on I_{DS} and I_{leak} . Continuous black line: I_{DS} DC sweep; continuous red line: I_{DS} pulsed-gate; dashed black line: I_{leak} DC sweep; dashed red line: I_{leak} pulsed-gate.

A schematic of all-plastic LGFET employed in this study is depicted in Figure 7.1 (a). The liquid sample is passed through the microfluidic channel and all biomolecular interactions are sensed by the SWCNT film in contact with the channel. Under normal DC sweep operation, the effective V_G that can be applied is limited only within $|V_G| < 1$ volt due to electrolysis of water.

All chemical reactions need a finite amount of time for completion; and in the case of electrolysis of water the required time is of the order of 1 ms ^[111]. The electrolysis reaction may thus be avoided even at high V_G values by applying a bias over a time-scale of less than 1 ms. Therefore, by applying a string of pulses (Figure 7.1 (b)) with frequency greater than 1 kHz, the electrolysis reaction can be avoided kinetically, and hence provide a way to extend the operating V_G window of the LGFET to $|V_G| > 1$ V.

A comparison between the I_{DS} - V_G obtained with pulsed-gate and simple DC sweep when the LGFET is operated within $-2 \geq V_G \geq -0.5$ V window is displayed in Figure 7.1 (c). As noted, the two curves appear similar when the bias applied is $|V_G| < 1$ volt in that the I_{DS} shows an uptrend as the V_G becomes more negative, indicative of p-type transistor behavior typical of SWCNT transistor reported in the literatures ^[73, 112]. However, the two curves starts to depart in direction when the $|V_G| > 1$ volt is applied. In particular, the I_{DS} - V_G curve obtained from simple DC sweep starts showing a downtrend as the V_G is increased further to $|V_G| > 1$ volt. This is in contrast with the I_{DS} - V_G obtained when the

pulsed-gate V_G is applied, which shows a persistent I_{DS} uptrend even at $|V_G| > 1$ volt. The dissimilarity of the two I_{DS} - V_G curves clearly demonstrates that the pulsed-gate V_G prevents the electrolysis reaction kinetically, and hence allows to extend the operating window of the LGFET to $|V_G| > 1$ volt.

In typical operation, a small amount of current usually travels through the dielectric, and constitutes the leakage current (I_{leak}) of the transistor. Normally, the magnitude of this I_{leak} is negligible in comparison to the I_{DS} ; however, the charge generation that results from the electrolysis reaction could lead tremendous increase in the I_{leak} of the LGFET when $|V_G| > 1$ volt is applied with simple DC bias. This large increment in I_{leak} which results from such reaction probably is the reason why the I_{DS} - V_G curve obtained from simple DC sweep exhibit a markedly different trend at $|V_G| > 1$ volt window than that obtained from pulsed-gate (Figure 7.1 (c)).

Additionally, in the operation of the LGFET as a biosensor, it is important to make sure that the pulsating V_G does not alter the device behavior. Hence, to check this aspect, I have also performed a kinetic measurement to examine the I_{DS} and the I_{leak} when both a pulsed-gate or DC sweep is applied, and the V_G is fixed constant (Figure 7.1 (d)). It can be observed that at a fixed V_G , neither the I_{DS} nor I_{leak} shows any significant difference which indicates that the device behavior is not altered even when a pulsating V_G is applied.

The protein interaction with the SWCNTs were investigated electrically, as shown in Figure 7.2 (a) which display the I_{DS} - V_G curve before and after exposure toward PLL when either simple DC sweep or pulsed-gate bias is applied. Here, it can be observed

that the I_{DS} - V_G is shifted toward negative bias direction after being exposed to the PLL, in both cases of simple DC sweep and pulsed-gate; and both techniques do not exhibit much change in the slope after the PLL exposure. A marked difference, however, is observed at high $|V_G| > 1$ volt regions whereby upon the PLL exposure, the pulsed-gate I_{DS} - V_G shows a decrease in saturation current (I_{sat}), commonly defined as the plateau in the I_{DS} - V_G curve; while such a change is absent in the I_{DS} - V_G obtained from simple DC sweep.

Figure 7.2 (a) I_{DS} - V_G before and after the nanotube exposure toward PLL. The threshold voltage in the graph is shifted toward negative V_G direction, indicative that the PLL is positively charged. (b) Kinetic data from simple DC sweep (black, V_G -0.5 volt) and pulse-gate (red, V_G -2 volt): (1) 0.2 aM, (2) 2 aM, (3) 20 aM, (4) 200 aM, (5) 2 fM, (6) 20 fM, (7) 200 fM, (8) 2 pM, (9) 20 pM, (10) 200

pM, (11) 2 nM, (12) 20 nM, (13) 200 nM, (c) Calibration plot from DC sweep (black) and pulse-gate (red). The application of pulse-gate leads to signal amplification and improvement in sensitivity as well as limit of detection.

Previous study ^[23] have shown that there are four different possible mechanisms in which biomolecules interact with the nanotubes: (a) electrostatic gating, (b) Schottky barrier, (c) capacitance change, and (d) mobility change. Each of these four possible interactions introduce a unique set of change in the I_{DS} - V_G curve ^[23]. Specifically, electrostatic gating causes a threshold voltage shift; Schottky barrier modulation results in a decrease in I_{DS} at $V_G < 0$, and increase in I_{DS} at $V_G > 0$; capacitance effect, on the other hand, results in a decrease in I_{sat} of the I_{DS} - V_G at negative and positive V_G ; and mobility changes (possibly scattering induced) cause a decrease in slope at both positive and negative V_G regions.

Hence, by analyzing how the I_{DS} - V_G curves (Figure 7.2 (a)) changes, information regarding the nature of interaction between the PLL and the SWCNTs can be obtained. The negative shift in I_{DS} - V_G observed in the figure for both simple DC sweep and pulsed-gate indicates presence of positive charge in the proximity of the SWCNT, which probably comes from the PLL as previously reported in the literature ^[23, 86]. Absence of significant change in the slope implies that the PLL adsorption does not lead to degradation of charge-carrier mobility on the nanotubes. The presence of I_{sat} decrease at high $|V_G|$ regions indicates that the capacitance of the LGFET is decreased as the PLL adsorbs on to the nanotubes. This decrease in capacitance is probably because most biomolecules usually have dielectric constant considerably lower than water. Hence, when significant number of PLL molecules is adsorbed on to the nanotubes surface, the

total capacitance in the LGFET is reduced, and leads to diminution of I_{sat} in the $I_{\text{DS}}-V_{\text{G}}$ curve. This modulation of I_{sat} is missing in the $I_{\text{DS}}-V_{\text{G}}$ obtained from the simple DC sweep because of its limitation to operate in $|V_{\text{G}}| < 1$ volt window.

In addition to $I_{\text{DS}}-V_{\text{G}}$ measurement, the LGFET is also able to perform real-time detection of biomolecular interaction with kinetic measurement ^[113], whereby in this case the V_{G} and V_{DS} are kept constant, and the I_{DS} is monitored as a function of time. In this view, the application of pulsed-gate can be regarded as a way to amplify the signal in kinetic measurement in that the I_{DS} drop at high $|V_{\text{G}}| > 1$ volt region appears larger than that observed at low $|V_{\text{G}}| < 1$ volt region (Figure 7.2 (a)). This discrepancy is rooted directly from the fact that the I_{DS} drop at high $|V_{\text{G}}|$ neighborhood is contributed from two factors: shift in the $I_{\text{DS}}-V_{\text{G}}$ curve, and change in the I_{sat} ; while the I_{DS} drop at low $|V_{\text{G}}|$ regime comes solely from the $I_{\text{DS}}-V_{\text{G}}$ shift. Indeed, the kinetic data in Figure 7.2 (b) clearly displays such amplification: the I_{DS} drop in kinetic data is evidently more prominent in the case of pulsed-gate (V_{G} -2 volt) than simple DC bias (V_{G} -0.5 volt).

Clearly, such amplification improves the sensitivity and the limit of detection (LOD) of the LGFET in a good way. The calibration plot obtained from triplicates of kinetic measurements for pulsed-gate and simple DC bias is shown in Figure 7.2 (c). The trend in the plot is fitted with three-parameter logarithmic regression function ^[105]:

Equation 7.2

$$\frac{\Delta I_{\text{DS}}}{I_0} = a - b \log([PLL] + c)$$

Correlation coefficient R^2 0.9 and 0.984 for simple DC bias and pulsed-gate, respectively implies that the regression equation fits the underlying data points quite well. The sensitivity for simple DC bias and pulsed-gate as referred from the slope of the plot are 0.058 and 0.262 % decade⁻¹, respectively. The standard error of the regression is taken as the square root of residual variance from the test of analysis of variance (ANOVA) [106] in the regression routine, which equals to 0.194 and 0.332 % for simple DC bias and pulsed-gate, respectively. The LOD of the LGFET for PLL detection in de-ionized water is then determined from 3σ criterion [72, 106], yielding values 0.1 fM and 0.1 aM for simple DC bias and pulsed-gate, respectively. Graphically it can be observed from Figure 7.2 (c) that the amplification of signal owing to pulse-gating leads to improvement of the sensitivity.

Figure 7.3 Simulated I_{DS} - V_G responses of the LGFET during PLL adsorption when only capacitance (a) or electrostatic gating (b) is affected, and when both: electrostatic gating and capacitance; are affected together (c). Note the similarity of features displayed here in figure (c) with the experimental result shown in Figure 7.2 (a). All I_{DS} - V_G curves at small V_G window $|V_G| < 1$ volt have been up-shifted for clarity.

Results from the simulation of the I_{DS} - V_G response during PLL adsorption are displayed in Figure 7.3(a)-(c), for cases when only capacitance (Figure 7.3 (a)) or electrostatic gating (Figure 7.3 (b)) is changed, and when both: electrostatic gating and capacitance; are altered (Figure 7.3 (c)). Similar to the result reported in the literature ³, when a biomolecular interaction leads only to a capacitance change, the threshold potential of the I_{DS} - V_G curve remains the same, but the I_{sat} is modulated. Such a modulation in I_{sat} is easily discerned if the V_G operating window is wide, and conversely may be not so obvious if the V_G operating window is small, as shown in Figure 7.3 (a).

This trend is in contrast with the case when biomolecular interaction leads only to electrostatic gating change, as revealed in Figure 7.3 (b). Here, the threshold potential is modulated as a result from the electrostatic gating change, and results in a shift of the I_{DS} - V_G curve, accompanied with a constant level of the I_{sat} . It can be anticipated from the figure that the change in threshold potential causes the entire I_{DS} - V_G curve to shift, such that the I_{DS} modulation appears constant throughout the simulated V_G bias.

Hence, when the biomolecular interaction leads to alteration of electrostatic gating and capacitance at the same time; the I_{DS} - V_G of the transistor displays a change of both I_{sat} and a shift concurrently, as shown in Figure 7.3 (c). Since a similar trend is observed in

this simulation when compared to the experimental data shown in Figure 7.2 (a), this gives a strong support that adsorption of PLL modulate both the capacitance and the electrostatic gating, of which the former is more predominant at high V_G bias, while the later is more prominent at smaller V_G window $|V_G| < 1$ volt. Additionally, the simulation result also supports the mechanism behind the signal amplification in kinetic measurements, as revealed in Figure 7.2 (b) in that the I_{DS} modulation at high V_G bias appears larger because of contribution from capacitance change as well as electrostatic gating, whilst the I_{DS} alteration appears smaller at low V_G bias, because it is originated mainly from the electrostatic gating.

Figure 7.4 (a) selectivity of the LGFET was examined by exposing the device toward anti-p53 and a non-pairing protein, such as BSA. (b) Calibration plot for detection of p53 in artificial saliva employing competitive immuno-assay protocol with tested concentrations of: (1) 0.1 fM, (2) 10 fM, (3) 1 pM, (4) 100 pM, (5) 10 nM, and (6) 1 μ M; with both pulsed-gating and simple DC bias. The

pulsed-gating and DC bias data points are different at 0.9 confidence levels as examined by a two-way ANOVA test.

In addition, the ability of LGFET to identify bioanalytes in real-life proxy situation is examined through detection of p53 head-and-neck cancer biomarker in 100x diluted artificial saliva in phosphate buffer ^[114-116]. Like most sensing technologies, the LGFET biosensor is required to demonstrate selectivity and sensitivity toward the bioanalytes. In this regard, the selectivity of the device can be examined by exposing the device toward anti-p53 and non-pairing protein, such as free BSA (Figure 7.4 (a)). As noted, the conjugated p53 probe molecule recognizes presence of anti-p53 in the solution and produces a drop in I_{DS} upon formation of immuno-complex. A non-pairing protein BSA, however, is incapable to form such immuno-complex with the p53 probe molecule, and hence its presence in the solution does not produce significant I_{DS} drop, as noted in Figure 7.4 (a). Such differential response of the device toward anti-p53 and BSA, hence, demonstrates the selectivity of the LGFET biosensor.

The sensitivity of the device was examined by exposure toward a series of p53 solution whose calibration plot resulted from the experiment is shown in Figure 4(b) which displays a comparison between pulsed-gating and simple DC methodology. As noted, the engagement of competitive immunoassay protocol yields a calibration plot which is inversely proportional to the concentration of the p53 in the sample. The signal amplification that is contributed from pulsed-gating in this case can be seen from the larger signal obtained at lower concentration levels which leads to improvement in the LOD from 10 nM (simple DC) to 10 fM (pulsed-gating), similar trend to previous

experiment with PLL. However, in contrast with the PLL experiment, a lower plateau appears at concentration 1 μM (simple DC) and 10 pM – 1 μM (pulsed-gating) which prevents the calibration plot reaching zero signal levels at high concentration, typical for calibration plot of competitive immunoassay. Presence of foreign proteins in the artificial saliva, along with higher ionic-strength, may contribute to such unusual behavior in the calibration plot, and warrants for further study. The attained LOD of 10 fM upon employment of pulsed-gating, however, is lower than the important clinical p53 concentration (~ 60 pM) ^[117] required in head-and-neck cancer screening, and hence highlight the potential of the LGFET for future application in medical setting.

7.3 Chapter Summary

In this report I have proposed pulse-gating as a method to broaden the operating window of the SWCNT LGFET beyond the electrolysis boundaries of $|V_G| < 1$ volt. The pulse-gating allows to extend the operating window of the LGFET by preventing the electrolysis reaction of water from taking place, hence preventing a strong I_{leak} and allowing for higher amplification of the I_{DS} signal. Results from the pulse-gating experiments and simulation show that the sensing mechanism in the LGFET is predominated by two factors: electrostatic gating and capacitance change; of which the later is observable only at high V_G window, accessible only with pulsating gate bias. Alternatively, the larger I_{DS} drop at high V_G regions can also be employed to amplify the signal, and hence pushes the sensitivity and detection limit of the LGFET down to attomolar concentration levels. The applicability of LGFET to detect p53 head-and-neck cancer biomarker in artificial saliva is also reported employing competitive immunoassay

protocol, and engaging simple DC and pulsed-gating methodology. Again, the application of pulsed-gating is observed to results in improvement of the LOD from 10 nM (simple DC) to 10 fM (pulsed-gating), which is lower than the critical medical concentration (~ 60 pM) for screening of head-and-neck cancer, and highlight the potential use of LGFET for future biomedical applications.

Chapter 8 Conclusions and Suggestions for Future Works

Many biomolecular interactions have electrostatic origin which is the basis for detection through electrical measurements without the need for a label. In this regard, the size similarity between nanotubes and most biomolecules, and special electronic properties determined by the hollow-tubular architecture; renders SWCNTs a natural candidate as transducer elements for highly sensitive detection of biomolecules through electrical measurements in LGFET format.

Since the operation is in aqueous medium, LGFETs are capable to detect biomolecular interactions in real-time and label-free fashion. There have been several reports on LGFETs sensing, however, up to date the sensing mechanism in nanotube transistor is still not fully understood. The final objective of this thesis has been to address this challenge. In this effort, the work in this thesis also includes: (1) the fabrication process and characterization of nanotubes based LGFET made in plastic, (2) the experimental and simulation studies of the interaction between the biomolecules and the SWCNTs, (3) the application of the LGFET for biomolecular sensing involving immunoassay reactions (4) fundamental studies for elucidation of the biosensing mechanisms, and (5) investigation of a suitable amplification strategy to improve the sensitive of the LGFET to the highest level.

This chapter summarizes the conclusion and original contribution that stem out from the extensive study that has been performed, as well as suggestions of the future work that can be carried out to further improve the device operation for detecting a wide range of

biomolecular events.

8.1 Conclusions

In this thesis SWCNTs based liquid gated transistor have been fabricated and studied for application in biosensing. In this framework, I have specifically investigated the interaction between the nanotubes and biomolecules through electrical measurements by taking advantage of the innate properties from the nanotubes, such as: size similarity between the SWCNTs and most biomolecules, and their all-surface architecture. The major conclusions that can be drawn from the series of experiments and simulations that have been performed throughout the course of this study can be highlighted in several points:

1. *Laminated SWCNT-based LGFET has a simple and robust fabrication method for production of transistor devices, which engages simply two materials, namely: SWCNTs and PDMS; and alleviates the need for photolithography process at all, beyond the requirement of the initial hard mold preparation*

As described in detail in Chapter 3, the fabrication of laminated LGFET based on nanotubes with integrated microfluidic channel is extremely simple compared to the traditional device design, which involves SWCNT grown on a Silicon substrate and patterning of gold electrodes. In contrast to this traditional approach, the laminated LGFET requires merely two materials, namely: SWCNTs and PDMS for preparation of the device. Additionally, the device architecture also eliminates the need for photolithography steps altogether, beyond the requirement of initial hard mold

preparation. Evidently, such simplification of the fabrication process is advantageous because it allows a high production rate of devices. Additionally, the integration of microfluidic channel in the device architecture offers advantages, such as: controlled delivery of the liquid sample, minute requirement of sample volume, and ability to perform kinetic measurements among others. The biomolecular interaction is then studied in real-time and label-free fashion within the aqueous liquid environment through either kinetic and/or transfer characteristic measurement.

2. *Interaction between the proteins and the nanotubes is influenced by the surface chemistry of the nanotube which determines specific conformational changes upon interaction and result in a distinct signal level in the LGFET.*

The ability of the LGFET to detect biomolecular events is originated from the interaction between the nanotubes and the biomolecules, which results in modulation of electrical properties of the former. In Chapter 4, it has been revealed that protein/nanotube interaction is influenced by the surface chemistry of the nanotube and has a reciprocate effect to both: the conformation of the protein as well as the conductance of the nanotubes; this effect is reflected in the signal level in the LGFET, Circular Dichroism and Fluorescence spectroscopy, and molecular-dynamic simulation.

3. *The SWCNT LGFET can be employed for detection of important bioanalytes, such as herbicide, and its sensitivity is influenced by the ionic-strength of the solution*

In Chapter 6, I have engaged lock-and-key model of immuno-complex formation to introduce selectivity in the LGFET toward herbicide 2, 4-D. The small size of the

analytes (220 g mol^{-1}) as compared to its antibodies ($\sim 150,000 \text{ g mol}^{-1}$), however, makes it impossible for the analytes to be detected in direct assay fashion. Hence, to circumvent this problem, a competitive assay needs to be employed in which free analytes are competing with the analytes/protein conjugate bound on the surface of the nanotubes, to combine the free antibodies. The competition yields a calibration plot which is inversely proportional to the concentration of the free 2, 4-D in the sample solution, and the high sensitivity nature of the nanotubes allows the device to attain detection limit of 50 pM and 500 fM in buffer and soil extract solution, respectively. The better detection limit observed in the case of soil sample can be attributed to the ionic strength of the solution, which is lower for the case of soil extract in comparison to that of buffer solution, supporting the result of studies described in Chapter 5.

4. *Pulsating gate bias extends the operating window of the LGFET beyond ± 1 volt electrolysis boundary with the implication of improving the detection limit down to atto-molar levels and highlighting the role of capacitance as sensing mechanism at higher bias regime*

The ability of nanotube to operate in aqueous medium brings an advantage: real-time detection of biomolecular interaction; and disadvantage: inherent DC gate bias limit to ± 1 volt due to electrolysis of water. This disadvantage, however, can be circumvented by applying a high frequency ($f > 1 \text{ kHz}$) pulsating gate bias which preempts the electrolysis reaction kinetically. As described in detail in Chapter 7, the pulsed-gating method allows one to study the effect of capacitance as sensing mechanism at high bias regime, as well as amplify the signal to attain detection limit down to atto-molar concentration levels.

The application of pulsed-gating also allows to detect presence of p53 head-and-neck cancer biomarker in artificial saliva with a detection limit down to 10 fM level, highlighting the potential of LGFET for uses in biomedical diagnostics.

8.2 Suggestion for Future Works

8.2.1 Improving the signal-to-noise ratio through instrumentation and electrical filter

Like in most biosensing technologies, one of the most important parameters for LGFET biosensors is sensitivity, which is influenced by the signal-to-noise ratio (SNR). Mathematically, the SNR is defined as

Equation 8.1

$$SNR = \left(\frac{A_{signal}}{\sigma_{noise}} \right)^2$$

Where A_{signal} and σ_{signal} are the amplitude of the signal and the standard-deviation of the noise, respectively.

From technical perspective, the SNR parameterizes the quality of a signal that comes from an instrument In the presence of Gaussian white noise. In typical practice, a SNR of three is generally considered as a ‘minimum safe limit’ to separate the signal from the noise. Hence, improvement of the quality of the signal in the future necessitates the improvement in SNR as well. From Equation 8.1, it is clear that there are two general approaches to enhance the SNR: (1) reduce the noise levels, and/or (2) amplify the signal.

Figure 8.1 General routes to attain higher SNR: (1) reduce the noise levels, and/or (2) amplify the signal. In the first approach, the SNR is enhanced through many filtering techniques available to date, both digitally and physically. In the later approach, the SNR is improved through techniques, such as: enzymatic amplification, tagging with nanotubes, silver deposition onto gold nanoparticles, cascading DNA to form a vast inter-linked network, just to name but few.

In the former approach, the reduction of noise levels can be attained by applying various digital and analog filters to the measurement system. In the later strategy, the SNR can be improved by amplifying the signal levels in the LGFET through various possible techniques, such as: (1) enzymatic activity, (2) employing SWCNT/biomolecule conjugate as amplifier agent, (3) application of silver enhancement on gold nanoparticle/biomolecule conjugate, among others.

8.2.2 Integration with other microfluidic technologies for realization of Lab on a Chip concept

The integration of microfluidic channels into the laminated LGFET design determines that the device can be categorized into microfluidic technology. In this framework, the transistor hence can be potentially integrated with other types of microfluidic devices for development of lab on a chip concept which aims to miniaturize and automate various processes in the laboratory on a single chip.

One example of in the microfluidic field is the application of microchannel to direct the fluid sample for formation of lipid bilayer on a SWCNT network in a variant liquid-gate transistor design ^[13]. In this case, the microchannel functions to direct the liquid sample to the active sensing area where the nanotube resides. The formation of the lipid bilayer can be monitored in real-time through LGFET kinetic measurement. The lipid bilayer can then be used to incorporate specific receptor proteins to determine analytes of interest such as drugs, biomolecules or bio-chemicals.

Figure 8.2 (a) microchannel attached on top of the nanotube network functions to control the delivery of liquid sample for formation of lipid bilayer, and (b) a schematic showing a lipid bilayer membrane formed on to a nanotube. The formation of the lipid bilayer membrane can be monitored in real-time by the LGFET kinetic measurement.

8.2.3 Exploration of other semiconducting materials to substitute the SWCNTs in LGFET

Alternatively, the LGFET can also be modified for customization toward a certain need in the experimentation. One of such modification can be done by substituting the nanotubes in the LGFET with other types of semiconducting materials, which can include:

silicon nanowires, organic semiconductors, graphene, etc. The substitution of these materials into the LGFET could enrich the variety of the device, and hence opens up a possibility to custom tailor the device for a plethora of applications.

For example, the substitution by graphene has been proven theoretically by Yan et al [118] which indicates that in theory graphene transistors should have a similar behavior to that of SWCNTs transistors, with some differences which stem from the fact that graphene has a planar instead of tubular geometry. Yan et al have also shown that the modification of the graphene transistor can be done by doping through functionalization of the ribbon edge, which is impractical in the case of nanotubes due to absence of such edge.

Figure 8.3 Graphene as a potential candidate material for substituting the SWCNT in LGFET device. Theoretical work by Yan et al [118] shows that graphene transistor displays a similar ambi-

polar behavior like the nanotube counterpart. However, in contrast with the nanotube, the graphene can be doped by changing the chemistry of the edge of the ribbon.

8.2.4 Integration of the LGFET with electronic devices for memory and various other electronic applications

In an alternative perspective, the LGFET can also be thought as an electrical device because of its functionality as a transistor. Hence, in this school of thoughts, the LGFET can also be integrated with other electronic components to form complete electronic circuits, such as: logic inverter, memory devices, ring oscillators, etc. Plainly, such integration could lead to wider applicability of the LGFET beyond simply sensing purposes.

One example of a similar expansion in application has been demonstrated by Bachtold et al ^[119] whereby a network of *dry-state* carbon nanotube transistors have been organized in various arrangement connected with traditional electronic components, such as resistors, to form logic circuits, such as: inverter, and NOR gate; SRAM, and a ring oscillator. The kernel of the idea plainly can also be extended to be applied in LGFET, i.e. connecting the liquid-gated transistor with various electronic components to form an integrated circuit network.

Figure 8.4 Electronic circuit formed from dry-state SWCNT transistor ^[119]. The kernel of the idea can be potentially extended for application with LGFET transistor whereby the transistor connected with various traditional electronic components to form an integrated electronic circuit.

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Science never solves a problem without creating ten more

George Bernard Shaw (1925 Nobel Laureate in Literature, 1856 – 1950)

List of Publications

Patent

(1). Flexible Laminated Microfluidic Integrated Device for Electronic Applications

Tey Ju Nie, Wang Zongbin, Alagappan Palaniappan, Subodh Mhaisalkar, I Putu Mahendra Wijaya, Isabel Rodriguez

PCT Patent Application, 19 September 2009

Journal Publications

(2). Protein/ carbon nanotubes interaction: the effect of carboxylic groups on conformational and conductance changes

I Putu Mahendra Wijaya, Sonu Gandhi, Tey Ju Nie, Nishima Wangoo, Isabel Rodriguez, G. Shekhawat, C. Raman Suri, Subodh G. Mhaisalkar

Applied Physics Letters **95**, 073704 (2009)

(3). Laminated, microfluidic-integrated carbon nanotube based biosensors

Ju Nie Tey, I Putu Mahendra Wijaya, Zongbin Wang, Wei Hau Goh, Alagappan Palaniappan, Subodh G. Mhaisalkar, Isabel Rodriguez, Simon Dunham, John A. Rogers

Applied Physics Letters **94**, 013107 (2009)

(4). Effect of alignment on the temperature-resistance response of dielectrophoretically assembled multi-walled carbon nanotube films

I Putu Mahendra Wijaya, Isabel Rodriguez, Subodh G. Mhaisalkar

International Journal of Nanoscience **7** (4 &5), 1 -7 (2008)

(5). Femto Molar Detection of 2, 4-dichlorophenoxyacetic acid Herbicides via Competitive Immunoassays in a Microfluidic Based Carbon Nanotube Liquid Gated Transistor

I Putu Mahendra Wijaya, Tey Ju Nie, Sonu Gandhi, Robin Boro, Alagappan Palaniappan, Goh Wei Hau, Isabel Rodriguez, C. Raman Suri, Subodh G. Mhaisalkar

Lab on a Chip, **10**, 634-638

(6). Investigation of sensing mechanism and signal amplification in carbon nanotube based microfluidic liquid-gated transistor via pulsating gate bias

I Putu Mahendra Wijaya, Tey Ju Nie, Alagappan Palaniappan, Isabel Rodriguez, Subodh G. Mhaisalkar

Lab on a Chip, (in press), DOI: 10.1039/b926631c

(7). Real-time immuno sensing of morphine using LG-CNT FET

J. N. Tey, S. Gandhi, I. P. M. Wijaya, I. Rodriguez, C. Raman Suri and S. G. Maisalkar

Small, accepted, 2010

(8). Microfluidic Integrated Nano-net Based Biosensors

J. N. Tey, I Putu Mahendra Wijaya, Alagappan Palaniappan, Isabel Rodriguez, S. G. Mhaisalkar

Micro and Nanofluidics (invited review article), accepted, 2010

Conference

(9). A Microfluidic All-Plastic Liquid-Gated Single-Walled Carbon Nanotube Field Effect Transistor for Immunoassay Detection of 2, 4-Dichlorophenoxyacetic acid Antibody in Aqueous Media

I Putu Mahendra Wijaya, Tey Ju Nie, Isabel Rodriguez, Subodh G. Mhaisalkar

Micro Total Analytical Systems (μ TAS), Jeju, Korea (2009)

(10). A novel all-plastic liquid-gated single-walled carbon nanotube field effect transistor for biosensing applications

I Putu Mahendra Wijaya, Ju Nie Tey, Isabel Rodriguez, Subodh G. Mhaisalkar

International Conference on Materials for Advanced Technologies (ICMAT), Singapore (2009)

(11). Conductance Surge During Dielectrophoretic Assembly of Carbon Nanotubes Into Temperature Sensing Film

I Putu Mahendra Wijaya, Isabel Rodriguez, Subodh G. Mhaisalkar

*International Conference on Materials for Advanced Technologies(ICMAT), Singapore
(2007)*