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One-Dimensional Conducting Polymer Nanostructures for Chemical and Biological Sensor Applications

A Dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Chemical and Environmental Engineering by Nicha Chartuprayoon

December, 2012

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ABSTRACT OF THE DISSERTATION

One-Dimensional Conducting Polymer Nanostructures for Chemical and Biological Sensor Applications

by

Nicha Chartuprayoon

Doctor of Philosophy, Graduate Program in Chemical and Environmental Engineering
University of California, Riverside, December 2012
Dr. Nosang V. Myung, Chairperson

Despite of their short history, conducting polymers such as polypyrrole (PPy) have emerged as a novel building block for label-free chemiresistive/FET chemical/biological sensors owing to a great environmental stability, active functional monomers for direct covalent immobilization of bioreceptors, remarkable optical, magnetic, and electrical properties like a semiconductor as well as mechanical property and ease of fabrication possessed by polymers. Tunable electrical conductivity can also be realized by several orders of magnitude via the process called ‘doping’ where anions or dopants is chemically or electrochemically incorporated to oxidized CP backbones to attain the charge neutrality.

Confining conducting polymers to one-dimensional (1-D) nanoscale has ultimately increased the surface area to volume ratio and facilitated the electron transport through the bulk of 1-D nanomaterials. Therefore, a small perturbation by adsorbed charged chemical/biological molecules on their surfaces significantly affected the charge distribution within the bulk of 1-D nanomaterials, enhancing the sensitivity and detection limits. While this nano-electronic chemical/biological sensor showed a
potential for advanced technology in detection and monitoring, device fabrication and assembly for 1-D conducting polymer nanostructures still appeared challenging to be scalable and reproducible in a cost effective manner.

Herein, this dissertation focused on fabrication and assembly of 1-D conducting polymer nanostructures based chemiresistive/FET chemical/biological sensors in a cost effective manner. First, lithographically patterned nanowire electrodeposition (LPNE) was used to batch-scale fabricate single PPy nanoribbon with controlled dimensions and defined location on various substrates for NH$_3$ detection. Various bioreceptors were also surface functionalized on LPNE grown PPy nanoribbon to investigate sensing performance in terms of sensitivity, selectivity, dynamic range, and detection limits towards the specific virus and the target protein. Polyclonal antibodies (pAbs) that recognized cucumber mosaic virus (CMV) were anchored on PPy nanoribbons for the detection of CMV. Single chain fragment variables (scFvs) specific for mycobacterium tuberculosis antigen 85 complex protein (Ag85) were functionalized on PPy nanoribbons to accommodate the electrostatic screening effect caused by dissolved salt concentration in buffer solution and diluted human serum. On the other hand, template-directed electrodeposition was employed to synthesize PPy nanowires using various dopants and solvents in order to evaluate the structure dependent sensing performances for detection of Ag85B protein.
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CHAPTER 1

INTRODUCTION: ONE-DIMENSIONAL NANOSTRUCTURES BASED BIO-DETECTION

Abstract

Herein, the recent application of one-dimensional (1-D) nanostructures (NS) based label-free chemiresistive/chemFET biosensors and the mechanism associated with the biomolecular detection was reviewed. The sensing performance in terms of sensitivity, selectivity, and reliability of chemiresistive/chemFET biosensors was also conferred based on the current sensor fabrication approaches, the surface functionalization schemes with the selection of bioreceptors, and the sensor operation. Finally the outlooks to chemiresistive/chemFET biosensors were recommended for a subsequent advancement in this field.
1.1 Introduction

Rapid, inexpensive and reliable biosensors for massive parallel sequences analysis of biological activities has been considerably researched as a promising analytical tool to numerous applications involving bio-terrorism, agriculture, pharmaceutical research, genomic and proteomic studies, and point-of-care (POC) diagnostics.\(^1\)\(^-\)\(^4\) A gamut of biosensor configurations including optical, piezoelectric, electrical and electrochemical biosensors prepared by planar thin films of metals, metal oxides, conducting polymers and other carbon materials as conventional transducers were developed and have been extensively investigated by many research groups.\(^5\)\(^-\)\(^8\) However, over the past decades, the advent of nanotechnology has prompted the integration of one-dimensional (1-D) nanostructures (NS) as transducers, which offered ample benefits. Miniaturized lab-on-chips (LoCs) using high density arrays of 1-D NS based nanodevices could be realized by various complementary innovative top-down fabrication and bottom-up synthesis along with assembly techniques.\(^9\) The ultra-high surface area to volume ratio, their comparable sizes to biomolecules, especially single-walled carbon nanotubes (SWNTs) where all atoms were on the surface, subtle structural properties, and electrical transport within the bulk of 1-D NS improved sensitivity and limit of detection possibly down to a single molecule detection.\(^10\) The response time was also enhanced by a virtue of the two-dimensional diffusion.\(^11\)

A great research effort on 1-D NS based bio-detection has been given particularly to optical, electrochemical, and electrical biosensors.\(^12\)\(^-\)\(^14\) In optical biosensors based 1-D NS, segmented metallic nanowires exhibited distinctive optical reflectivity to their adjacent segments were used as barcodes to enable a variety of recognition patterns. This alleviated the limitation of encoding or identifying large
numbers of parallel biological analyses owing to inadequate number of fluorophores that displayed different fluorescences. Strano and coworkers also pioneered the use of carbon nanotubes as robust labels for near-infrared (IR) optical sensors and solution-based in-vivo biological imaging, since they could endure long-term excitation without photobleaching and sustain chemical staining. This sensor configuration, despite several advantages, may not be practical to cost-effective portable analytical devices caused by expensive optical instrumentations that hampered the full exploitation of this technology in the field operation. Interfacing the biological activities with 1-D NS based nano-electronics therefore had a great potential to achieve the demand for portable biosensors in terms of low cost associated with sensor fabrication, sensor operation as well as ease of sensor miniaturization.

Electrical transduction of 1-D NS towards biological activities such as electrochemical reactions of biomolecules, antibody-antigen interactions or catalyzed enzymatic reaction was accomplished by several sensor designs and configurations that eliminated the use of chemiphores and fluorophores. 1-D NS functioned as a working electrode in electrochemical sensors illustrated exceptional sensing performance towards detection of biomolecules due to their inherited high surface area and a fast electron transport. The sensor response time was remarkably enhanced because of improved mass transfer caused by non-planar radial diffusion as compared to planar diffusion of macroscale microelectrodes. The limit of detection of 1-D NS based electrochemical sensors such as CNTs was achieved down to 160 zmol for proteins and 1.3 zmol for DNA, respectively. Another promising approach for direct and label-free electrical detection was chemiresistive/chemFET biosensors, which solely relied on the modulation of the electrical conductivity/resistivity of 1-D NS between microelectrodes.
Since the pioneer of 1-D based FET biosensors by Cui et al. in a decade\textsuperscript{21}, this sensor configuration has been increasingly explored as an excellent promise to inexpensive, miniaturized and reliable sensing devices for field operation.

Herein, this review will highlight the recent application of 1-D NS based label-free chemiresistive/chemFET biosensors and the mechanism associated with the biomolecular detection. In addition, the sensitivity, selectivity, and reliability of chemiresistive/chemFET biosensors will be reviewed based on current material synthesis and fabrication approaches, the surface functionalization schemes with selection of bioreceptors, and the sensor operation. Finally, the outlook to chemiresistive/chemFET biosensors will be recommended for subsequent advancement in this field.

1.2 Chemiresitive/chemFET biosensors using 1-D NS

1.2.1 Sensor configurations

Tremendous advancement towards reagent-less chemiresistive/chemFET biosensors has been realized by the utilization of semiconducting nanowires, nanotubes, nanobelts, and nanoribbons as 1-D conduction channel comprising of SiNWs, single-walled carbon nanotubes (SWNTs), conducting polymers (CPs) as well as metal oxides nanowires.\textsuperscript{10,22-24} Their diminutive diameter with high aspect ratio enabled facile fabrication and assembly of high density nanoscale sensor arrays by means of conventional well-developed microfabrication technology, while their high surface area to volume ratio and comparable sizes to biomolecules gave rise to high response towards minute perturbation from charged molecular adsorption and/or interaction on their surfaces.\textsuperscript{25} In this case, a charge accumulation/depletion region was generated in the
bulk of 1-D NS as compared to planar surface observed in conventional 2-D thin films, resulting in a significant electrical conductance/resistance change instead of a negligible change in thin films owing to an inherent lateral current shunting around the charge accumulation/depletion region as depicted in Figure 1.1. Consequently, the sensitivity and limit of detection of 1-D NS based chemiresistive/chemFET were substantially improved.

A chemiresistive biosensor shown in Figure 1.2A was the simplest sensor configuration, which measured the conductance/resistance of 1-D NS bridging across two terminal source (S) and drain (D) microelectrodes. The interaction between target analytes and bioreceptors immobilized onto 1-D transducers' surfaces or simply charged biomolecular adsorption onto 1-D transducers' surfaces induced an electrostatic effect that directly impacted their electrical characteristics. Xie et al. compared the sensing performance of aptamers modified bioaffinitive chemiresistors using PEDOT nanowires and PEDOT thin film for thrombin detection, and showed the substantial change in electrical conductivity of PEDOT nanowires upon thrombin binding (1 nM to 1 µM) as to negligible electrical conductivity change seen in PEDOT thin film. This corroborated the unique feature of 1-D NS as a novel sensing platform for chemiresistive biosensors. Additionally, this type of biosensor was not limited to sense only charged molecules based on bioaffinity principle or sensitive molecular interactions between 1-D NS and molecules themselves, but it allowed probing the small or uncharged molecules by employing a displacement immunoassay format in place of the affinity based detection as illustrated by Cella et al. and Park et al. for ultrasensitive glucose detection (nM range) and nitroaromatic explosives monitoring (sub-ppb range) with high sensitivity, respectively.
Arranging 1-D NS in FET formats augmented the sensitivity of chemical and biological sensors. It is comprised of three-terminal configuration: source (S), drain (D), and gate (G). Two common 1-D NS based FET sensor formats were exploited and termed back-gated FET and ion-gated FET (IGFET) shown in Figure 1.2B and 1.2C. In back-gated FET, not only was the electrical resistance/conductance affected by external charges upon biomolecular binding events, but it was also modulated by an applied gate potential through insulating layers such as SiO$_2$ and Si$_3$N$_4$ which served as a gate capacitor, thereby improving the sensitivity and detection limit of bio-detection. This sensor normally operates in air to mitigate the uncontrollability of the surface potential near 1-D NS due to the lack of a reference electrode in the solution. Nevertheless, in comparison with the conventional biosensors, the sensor should be capable of operating in a liquid environment. Therefore, IGFET became ideally suited. It composed of 1-D semiconducting NS served as a conductance channel contacted by S-D microelectrodes and a reference electrode functioned as a gate electrode in contact with the solution. In contrast to back-gated FET, the interfacial solution double layer between the solution and 1-D NS behaved as the gate capacitor which can tune channel conductance by applied potential at a reference electrode. Upon target-bioreceptor binding, shift in threshold voltage is observed because of the variation in capacitance within the interfacial double layer as observed by Heller et al.

### 1.2.2 Sensing mechanism

The fundamental understanding of sensing mechanism was a key in fabricating reliable and sensitive chemiresistive/chemFET biosensors. Modulation of schottky barrier height (SBH) and the electrostatic gating effect became dominated and mostly
addressed sensing mechanisms which held the distinctive gate dependent electrical characteristics.\textsuperscript{32}

For the sensing mechanism derived from schottky barrier height modulation (SBH), the initial SBH was governed by the work function different between metal-semiconductor contacts and the interface states.\textsuperscript{33} The local metal work function and thus SBH changed upon molecular adsorption led to a rearranged electronic band alignment. Modulation of SBH or the interfacial contact effect was mainly investigated in SWNTs based chemiresistive/chemFET biosensors in which the conductance/resistance decreased even if negatively charged molecules bound onto the sensors.\textsuperscript{34} This observation was not supported by charged biomolecules induced electrostatic gating effect as in the case of SiNWs based chemiresistive/chemFET biosensors. Chen et al. proposed that charged biomolecules caused modulation of SBH at the interfacial metal-semiconductor contacts was a dominating sensing mechanism from an investigation of streptavidin adsorption on SWNTs bridged across both of passivated and non-passivated microfabricated metal contacts.\textsuperscript{35} The unique IGFET characteristic of modulation of SBH imparted that the current transport was affected by a change in carrier mobility as opposed to that in carrier concentration observed in electrostatic gating effect.\textsuperscript{32} Thus far, no one has systematically proved the possibility to fabricate the reliable sensor operating in this sensing mechanism owing to the interfacial contacts that can be affected by many issues such as the impurity, and adhesion.

On the other hand, electrostatic gating effect has become predominating and reproducible sensing mechanism when charged molecules adsorbed onto the surface of 1-D semiconducting NS bridged S-D microelectrodes. Binding of negatively charged biomolecules on the p-type semiconducting channel gave rise to an accumulation of
charge carriers (i.e., holes) under the bound target analyte, thereby leading to an increase in channel conductance. Alternatively, binding of positively charged molecules would bring about a depletion of charge carriers, lowering the p-type channel conductance. In this case, the resulting conductance change of 1-D NS was proportionate to the change of their charge distribution upon molecular adsorption. Heller et al. investigated the drain current-gate potential characteristic of electrostatic gating effect sensing mechanism using SWNTs based IGFET and resulted in a shift in threshold voltage upon protein adsorption. This phenomena was relevant to a charge carrier redistribution.

Many research groups attempted to investigate the size dependent on the sensitivity of 1-D NS based chemiresitive/FET nanosensors. Elsform et al. investigated the electrical characteristics of pH sensor based SiNW FET with respect to various SiNW diameters ranging between 50-170nm and suggested that the sensitivity of SiNW FET towards exposure of different pH solution increased with a decrease in SiNW diameter. Interestingly, SiNWs with diameter exceeding 150nm behaved as Si wires with micron size in diameter. The surface charges resulting from protonation/deprotonation were not sufficient to impact the carrier redistribution within the bulk of SiNWs. Kim et al. also investigated the sensing performance of monoclonal antibody anti-PSA modified n-type SiNW FET with various channel width and length but constant doping density on the order of $10^{18}$ cm$^{-3}$. The smaller width with the longer channel exhibited higher resistance (i.e., conductivity below 500 nS) and was easily depleted by negatively charged PSA (pH = 6.9) in buffer solution (pH = 7.6), showing a detection limit as low as 30 aM.
The binding/interaction of target analyte-bioreceptor generated charge accumulation/depletion region normally termed debye screening length ($\lambda_D$) within the bulk of 1-D NS, which defined by

$$\lambda_D = \left(\frac{\varepsilon_r \varepsilon_0 k_B T}{p e^2}\right)^{\frac{1}{2}}$$  \hspace{1cm} (1)

Where $\varepsilon_r$ is dielectric constant of 1-D nanostructure, $\varepsilon_0$ is the vacuum permittivity constant (F/m), $k_B$ is boltzmann constant (eV/K), $T$ is temperature (K), $p$ is carrier concentration ($m^{-3}$), and $e$ is elementary charge (C). From this expression, $\lambda_D$ within 1-D NS is inversely proportional to $(1/p)^{1/2}$. Therefore, in the fabrication and assembly of high sensitive chemiresistive/chemFET biosensors using 1-D NS, some factors should be considered. First, the dimension of 1-D NS must be comparable to $\lambda_D$, especially for a chemiresistive sensor where there was no external gate to modulate the device conductance. Low carrier concentration must also be achieved in order to fully accumulate/deplete the carriers once molecular interaction took place near the surface of 1-D NS.\textsuperscript{36} In addition, the conductance of 1-D NS should be remarkably greater than that of the buffer solution to ensure a negligible leakage current through the solution which potentially introduced a significant device to device variation.\textsuperscript{39} This implied that conductance channel should have high mobility to compensate low doping concentration, or multiple 1-D NS based device with controlled spacing should be deployed.

Figure 1.3 illustrated the relationship between $\lambda_D$ vs. carrier density of semiconducting materials used in chemiresistive/FET sensors (black linear line) along with the tunable carrier density range (blue) and the smallest dimension that current technology enabled synthesis to date (red). The intersected area underneath the black linear line of each semiconducting materials including Si, SWNT, conducting polymers,
and metal oxides corresponded to the feasibility to fabricate highly sensitive chemiresistive/FET sensors. Besides dimensions and doping concentrations of 1-D NS, other factors significantly influenced the device performance as well. Table 1.1 summarized the materials’ properties entailing their dimensions, electrical properties, hydrophobility/hydrophilicity, and their stability in the physiological solution. Metal oxides were generally instable in physiological solution\textsuperscript{93}, and therefore they may not be compatible for chemiresistive/FET biosensor where the surface of conduction channel was exposed to a solution. By all the conditions discussed above, Si, SWNT, and conducting polymers became materials of choice. The detailed discussion of material synthesis, device fabrication, surface functionalization with various bioreceptors, and the sensing performance in terms of sensitivity, selectivity, and reliability of chemiresistive/FET biosensors will be reviewed.

1.3 Sensor fabrication and assembly

Small dimension and low carrier concentration were keys to sensing a minute effect induced by chemical and biological interactions. Highly sensitive and reliable 1-D NS based chemiresistive/chemFET biosensor arrays therefore necessitated well-developed synthesis methods with controlled dimensions, crystallinity, carrier mobility, and carrier concentration as well as massive sensor array integration processes for scale-up manufacture. Thus far, fabrication and device integration methods for 1-D NS based electronic devices were varied ranging from top-down approaches exploiting lithography for patterning, cutting and etching to bottom-up approaches where 1-D NS were formed by atomic arrangement.
Nanopatterning techniques via top-down lithography approaches such as electron beam lithography (EBL) technique generally facilitated the manipulation arrays of 1-D NS with an accurate positioning on substrates and a well control over material geometry, which was critical to the sensitivity of chemiresistive/chemFET devices. Donthu et al. illustrated the formation of polypyrrole (PPy) nanowire arrays by EBL patterning on spin coated polypyrrole film in the substrates, exhibiting the width, height, and nanowire spacing of 190nm, 100nm, and 2µm, respectively. Arrays of SiNWs fabricating from silicon-on-insulator (SOI) wafers using EBL followed by etching process typically had a width of 50nm or higher. This etching process generally produced defects, which deteriorated the high mobility of SiNWs. Stern et al. alleviated this challenge by chemical etching the patterned SOI wafers with tetramethylammonium hydroxide (TMAH) to produce a defect-free SiNW with retained high mobility. Coupling EBL with electrodeposition methods also promoted the selective growth of 1-D NS onto e-beam lithographically defined channel, in which the nanowire geometry was controlled by pre-defined EBL patterns. A site-specific electrodeposition of 1-D NS into the pre-defined channels supported the fabrication of high-density multiplexed sensor arrays in the miniaturized microchips.

Aside from utilization of EBL as nanopatterning technique, fabrication of 1-D NS based chemiresistive/chemFET sensors was also realized by other nanopatterning methods including dip-pen nanolithography (DPN) as well as superlattice nanowire pattern transfer (SNAP). As compared to EBL, DPN was a bottom-up approach that employed scanning AFM tip coated with an ink to directly and accurately pattern materials on the substrates both sequentially and in parallel with a high spatial resolution as shown by Lu et al. that reported the synthesis of poly(ethylenedioxythiophene)
(PEDOT) nanowires on a glass substrate for nitric oxide gas sensing. Well-aligned high density arrays of SiNWs with ultra-small width (10-20 nm) and pitch (20-60 nm) and 1 mm in length was fabricated directly on the substrate by SNAP. The accurate positioning resulting from these nanopatterning approaches subsequently enabled microelectrode contacts without complicated alignment processes involved.

Although these nanopatterning techniques became more viable due to the availability of SOI wafers and advanced micro and nanofabrication technology, the operating cost and serial process nature especially EBL still impeded the scalability of sensor fabrication. Bottom-up synthesis approaches thus showed high potential for a massive sensor fabrication. Template-directed chemical/electrochemical synthesis using both soft and hard templates was one of the well-known and cost-effective methods for bulk fabrication of various 1-D NS by controlling the synthesis parameters. Martin and coworkers first demonstrated the use of hard template-directed chemical/electrochemical polymerization to prepare polyaniline (PANI), poly(3-methylthiophene) (P3HT), and PPy nanowires by controlling polymerization conditions. Conducting polymer nanotubes were also formed when preferential nucleation initiated, and polymer elongated on the sidewalls of the templates owing to hydrophobic/hydrophilic interaction between oligomers and the sidewall of the membrane.

Randomly dispersed 1-D NS from template-directed chemical/electrodeposition method or even SWNTs suspension solution required subsequent alignment processes to position them onto pre-fabricated microelectrodes. Bangar et al. indicated versatile schemes to device functional PPy nanowire based ammonia electrodeposition of segmented Au-PPy-Ni-Au nanowires. Ferromagnetic Ni segment was selected to
facilitate magnetic alignment across the electrode gap, where Au segment ensured a good electrical contact.\textsuperscript{50} AC dielectrophoretic alignment was another alignment technique to assemble 1-D NS including conducting polymers as well as SWNTs between microelectrodes.\textsuperscript{2,22,29,51} Unfortunately, the CP nanowires bridged on top of electrode pads imparted poor electrical contacts. Thus, maskless electrodeposition method which selectively electrodeposits Au on Au electrode embedded CP nanowires, improving electrical contacts and mechanical joints.\textsuperscript{52}

In the case of SiNWs based array devices, the bottom-up approaches such as chemical vapor deposition (CVD) was established by Lieber’s group, which relied on vapor-liquid-solid (VLS) mechanism to grow single crystalline SiNWs.\textsuperscript{53,54} Bulk synthesized SiNWs exhibited a good control over selective doping, with a diameter as small as 10nm, length up to 1mm via the size of catalyst nanoparticles used (i.e., Au nanoparticles) and well controlled reaction parameters, respectively.\textsuperscript{54} This growth strategy brought in randomly oriented SiNWs, in which a fluidic alignment technique facilitated parallel NW arrays formation, followed by photolithographically defined microelectrodes for device integrations, depicting high potential to scalable fabrication.\textsuperscript{55} Recently, Lieber’s group fabricated phospholipid bilayers immobilized kinked SiNW nanoscale FET. This device was inserted into an HL-1 cell and used to record the intracellular responses by measuring the difference in electric potentials.\textsuperscript{56}

Alternatively, a template-free electrodeposition technique offered a simple route to selectively grow nanowires/nanofibers directly onto the pre-fabricated without expensive EBL and other instruments involved. Tseng and coworkers illustrated site-specific electrochemical polymerization of conducting polymer nanowires junction arrays (CPNEJs) between pre-fabricated microelectrodes which resulted in a cluster formation.
of nanowires that possibly produced a scattering site at the inter-contact between adjacent nanowires. Template-free electrodeposition technique using AC electric field enabled synthesis of oriented arrays of P(EDOT-COOH) nanowires bridging the micro-fabricated electrodes. Radical cations of oxidized EDOT-COOH produced at the anode migrated to cathode by external electric field across the electrode gap, and nanowires were formed. Formation of nanowires reduced the local electrical field, preventing nearby growth of nanowires. In addition to bottom-up growth methods for conducting polymers and SiNWs, the interesting structure-property relationship of SWNTs instigated large-scale selective growth process of SWNTs based electronic devices with less impurity of catalyst residues and carbonaceous particles. Aligned SWNTs arrays were selectively synthesized onto patterned catalyst microelectrodes under an applied electric field.

Additionally, the combination of top-down and bottom-up approaches significantly improved massive fabrication and device integration. Recently, Penner and coworkers have developed lithographically patterned nanowire electrodeposition (LPNE) to synthesize arrays of nanowires with rectangular cross-section to pre-defined locations on various substrates. This technique also provided feasibility to control the geometry of nanowires; the thickness and width of nanowires were independently tailored via thermally deposited sacrificial layer and electrodeposition parameters, respectively. We have also demonstrated the feasibility of this synthesis method to fabricate wafer-scale of ultra-long (>1cm) PPy nanoribbons with integrated microelectrodes to pre-determined position on 4” oxidized Si wafers and flexible polyimide film. Recently, the feasibility of this fabrication technique with the biocompatibility of conducting polymers, engineered
M13 bacteriophage-specific antibody were electrochemically entrapped during electropolymerization of PEDOT nanowires.\textsuperscript{61}

1.4 Factors impacting sensing performance

Apart from the importance of material properties and geometries to the sensing performance of 1-D NS based chemiresistive/chemFET biosensors, several other factors strongly impacted the sensing performance such as the effect of molecular charge screening by dissolved solution counterions on the sensor response, also known as the Debye screening effect.\textsuperscript{62} Practically, label-free chemiresistor/chemFET biosensors detected and responded to change in a distribution of charge carriers of 1-D NS from an electric field induced by interaction of charged chemical/biological substances near its surface or an electrostatic potential. However, owing to electrostatic interactions in the electrolyte-based solution, the charged biomolecules were screened by dissolved counterions, giving rise to a charge screening effect that generated an exponential decay of the electrostatic potential as the distance was away from the surface.\textsuperscript{62} The maximum distance at which the electrostatic potential from external charged molecules affected the distribution of charge carriers in 1-D NS, defined as Debye screening length or solution double layer ($\lambda_D$), was given by

$$\lambda_D = \frac{1}{\sqrt{4\pi l_B \sum_i \rho_i z_i^2}}$$  \hspace{1cm} (2)

Where $l_B$ is the Bjerrum length = 0.7nm for an aqueous solution, $\rho$ and $z_i$ are density and valence of ion species $i$, respectively.\textsuperscript{62} Hence, selection of the electrolyte solution strongly impact the sensitivity of the biosensors. In general, 1-D NS chemiresistive/chemFET biosensors were operated in low salt concentration medium to circumvent the electrostatic screening effect by dissolved counterions.\textsuperscript{21,24,31} Stern et al.
corroborated the importance of the Debye screening length by showing the streptavidin sensing using biotin functionalized SiNWs device at different concentration of PBS buffer solution. Sensing in the presence of diluted PBS buffer solution (0.01X) revealed the highest sensitivity.\textsuperscript{62} Selective target binding could also be enhanced by selecting an appropriate buffer conditions such that the non-selective binding was screened out.\textsuperscript{62}

Another factor that limited the sensitivity of 1-D NS based chemiresistive/chemFET biosensors was the surface functionalization schemes. In particular to SiNWs, the presence of the oxide layer was terminated by hydrophilic hydroxyl group that could undergo silanization with alkoxy silane derivatives such as 3-(trimethoxysilyl)propyl aldehyde (APTMS), 3-(aminopropyltriethoxysilane) (APTES), and 3-mercaptopropyltrimethoxysilane (MPTMS) to generate active functional amine, thiol, or aldehyde groups on Si/SiO\textsubscript{2} nanowires for a subsequent covalent attachment of bioreceptors.\textsuperscript{21,24,63} The native surface oxide and additional siloxane glass formation substantially decreased the sensitivity and detection limits, since the oxide surface was served as a dielectric that screened the external charges induced by molecular interactions. The surface oxide was chemically treated by dilute HF acid or buffer oxide etchant (i.e., HF and NH\textsubscript{4}F solution), resulting in hydride terminated surface (Si-H) that undergone photohydrosilylation in the presence of UV light with olefin derivatives such as 10-N-Boc-amino-dec-1-ene.\textsuperscript{64} Comparing to oxidized SiNWs towards detection of DNA, the detection limit of alkylated non-oxidized SiNWs was improved from 1 nM to 10 pM, and the dynamic range spanned by 100 folds.\textsuperscript{64}

In the case of SWNTs based chemiresistive/chemFET biosensors, non-covalent attachment of the bi-functional linkers such as the pyrene derivative moieties were highly preferred and became a generalized method over surface biofunctionalization via direct
covalent immobilization to functionalized SWNTs.\textsuperscript{65} Modification of the SWNTs sidewalls generated defects which lessened the opto-electronic properties of the pristine SWNTs.\textsuperscript{66} Non-covalent attachment of bi-functional linkers, although preserved the intrinsic properties of pristine SWNTs, caused the molecular interactions taking place further away from the surface as seen in the case of SiNWs. In addition to surface functionalization, synthesis and purification methods of SWNTs to produce intrinsic semiconducting SWNTs were essential to develop ultra-sensitive SWNTs based chemiresistive/chemFET biosensors. Recently, Ishikawa demonstrated the fabrication of the sensitive and reliable devices by tuning the number of nanotube densities.\textsuperscript{67} Low density SWNTs based biosensor devices imparted the detection limit of 5 nM towards SARS biomarker protein monitoring at the physiological solutions. This was attributed to semiconductor-like behavior of the low density of carbon nanotube network in which the electrical properties strongly depended on the gate potential and low capacitance.\textsuperscript{67}

On the other hand, the versatility of surface immobilization schemes, various monomer chemistries, their biocompatibility, and ease of fabrication prompted the use of conducting polymers as an emerging class of electrical transducer. Various surface biofunctionalization approaches were established and categorized mainly to an entrapment, physical adsorption, cross-linking, and covalent attachment which promoted the molecular interactions closer to the conducting polymer surface.\textsuperscript{22,42,68}

An additional factor that limited the sensitivity of label-free bioaffinity-based chemiresistive/chemFET biosensors was the bioreceptor used to capture target analytes. Although sensitive, selective, and multiplexed detections employing antigen-antibody interactions have been illustrated\textsuperscript{69}, these sensors which operate in a low ionic strength might not be feasible in physiological conditions owing to the electrostatic
screening effect caused by large amount of salts in a biological medium.\textsuperscript{62} Therefore, smaller bioreceptors such as aptamers, single-stranded DNA, peptide nucleic acid (PNA) and single chain fragment variable antibodies (scFv) have been suggested to ensure that the biological interaction occurred within the thickness of a Debye screening length (i.e., solution double layer) of liquid electrolyte.\textsuperscript{70}

Table 1.2 below summarized the properties of various bioreceptors employed for chemiresistive/chemFET biosensors. Despite high specificity to immunogenic targets, the typical size of antibodies was roughly 10nm, exceeding solution double layer thickness associated with the physiological solutions. Thus, single chain fragment variable antibodies (scFvs) were proposed overcome this challenge owing to their reduced dimension (i.e., ~3nm), but they retained the specific binding affinity towards target analytes. scFvs typically synthesized by genetically engineering instead of in vivo biological system as in synthesis of antibodies. Lo et al. constructed oriented anti-CEA scFvs immobilized on Ni decorated SWNT FETs for CEA detection, imparting low detection limit of 4pM.\textsuperscript{71}

In addition, aptamers, whose size was approximately 2nm, have also been widely used as bioreceptors. They were synthetic oligonucleotides exhibiting highly specific recognition to different targets such as ions, small organic molecules, proteins, and whole cells.\textsuperscript{70} Aptamers were isolated through repeated rounds of in-vitro selection, namely SELEX, leading to cost-effective mass production. Another advantage was stable to long term storage as compared to antibodies which have limited shelf-life owing to sensitivity towards temperature and denaturation when in contact with surface.\textsuperscript{72} Maehashi et al. exploited label-free aptamer-modified SWNT FETs for IgE detection. The sensors imparted the detection limits of 250pM, comparing to undetectable signal
observed when employing IgE-mAb modified SWNT FETs at the same concentration. This finding corroborated the sensing performance was affected by solution double layer and size of bioreceptors.

Label-free DNA based chemiresistive/chemFET sensors have become intensively researched, since DNA was very stable and could be regenerated for repeated use. A synthetic single stranded DNA oligomer (ssDNA) comprised of short oligomers of 10-40 mers was normally employed as the bioreceptor immobilized on 1-D nanotransducer. The nucleic acid hybridization relies on DNA base pairing between ssDNA and its complementary target. Direct electrical signal upon hybridization can be procured that greatly reduce the traditional DNA assay time. High specificity of DNA hybridization can be achieved via discrimination of single mismatch DNA base pair.

Since first described by Nielsen’s group in 1991, peptide nucleic acid (PNA) has emerged as a powerful bioreceptor that specifically hybridized to complimentary DNA targets. Instead of phosphate backbone, PNA’s backbone comprised of repeating N-(2-aminoethyl)glycine units linked by peptide bonds where the purine and pyrimidine bases were conjugated to backbone by methylene carbonyl bonds. Thus, PNA can be envisioned as charge neutral molecules. PNA-DNA interactions as a result exhibit stronger interaction than that of DNA-DNA due to the lack of electrostatic repulsion induced by charged phosphate groups. The intriguing feature remarkably enhanced sensitivity towards DNA hybridization. The carrier density of 1-D nanostructures can be influenced solely by charged complementary DNA.

The sensor operating parameters also played a significant role in sensitivity enhancement of 1-D NS based chemiresistive/chemFET biosensor. Gao et al. reported PSA detection using monoclonal antibody anti-PSA modified p-type SiNW FET in which
SiNW has a diameter of 20nm and the doping concentration on the order of $10^{18} - 10^{19}$ cm$^{-3}$, leading to a Debye screening effect ($\lambda_D$) of 2 nm.$^{77}$ This sensor showed limit of detection of 750fM. The sensitivity and limit of detection could be further improved if the charge carriers were fully accumulated under the binding region. Therefore, the sensor was operated in the sub-threshold regime where the surface charge from biomolecules exponentially changed device conductance as compared to linearly changed device conductance. By operating SiNW FET in this regime, limit of detection towards PSA decreased extensively to 1.5fM.$^{77}$

Another significant issue to enhance the sensing performance was the selectivity. Non-specific binding (NSB) of non-target molecules to bioreceptors impeded the sensitivity as well as low detection limits of label-free 1-D NS based chemiresistive/chemFET biosensors. The generalized method used chemical and biological molecules exhibiting a hydrophilic nature such as ethanolamine or bovine serum albumin (BSA) to block the remaining active sites on the surface of the biosensors.$^{2,21}$ Star’s group also coated the mixture of two polymers: poly(ethylene imine) (PEI) and poly(ethylene glycol) (PEG) functioning as a linker to a covalent coupling of biotin-N-hydroxy-succinimidyl ester and NSB blocking, respectively.$^{10}$ Furthermore, simultaneous complementary detection of target molecules using both n-type and p-type semiconductors were realized to rule out the false signal associated from NSB.$^{69}$
1.5 Research objectives

Despite of their short history, conducting polymers such as polypyrrole (PPy) have emerged as a novel building block for label-free chemiresistive/FET chemical/biological sensors owing to a great environmental stability, active functional monomers for direct covalent immobilization of bioreceptors, remarkable optical, magnetic, and electrical properties like a semiconductor as well as mechanical property and ease of fabrication possessed by polymers. Tunable electrical conductivity can also be realized by several orders of magnitude via the process called ‘doping’ where anions or dopants is chemically or electrochemically incorporated to oxidized CP backbones to attain the charge neutrality.

Confining conducting polymers to one-dimensional (1-D) nanoscale has ultimately increased the surface area to volume ratio and facilitated the electron transport through the bulk of 1-D nanomaterials. Therefore, a small perturbation by adsorbed charged chemical/biological molecules on their surfaces significantly affected the charge distribution within the bulk of 1-D nanomaterials, enhancing the sensitivity and detection limits. While this nanoelectronic chemical/biological sensor showed a potential for advanced technology in detection and monitoring, limitation in device fabrication and assembly for 1-D conducting polymer nanostructures appears challenging to be scalable and reproducible in a cost effective manner.

The overall objective of this work is to fabricate and assemble 1-D Polypyrrole (PPy) nanostructures based label-free bioaffinity chemiresistive biosensors and investigate the factors that influence their sensing performance for sensor optimization with particular applications to the detection of plant pathogens and protein markers. The overall goal will be achieved by the following:
1.) To explore the possible enhancement of sensitivity by
   a. Tailoring the dimension and charge carriers of 1-D PPy nanostructures
   b. Employing bioreceptors with different sizes and binding affinity, and
   c. Using buffer solution with different ionic strength
2.) To explore the possible enhancement of longevity and NSB by electrochemically synthesizing PPy nanowires with various dopants and solvents
1.6 Reference


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Figure 1.1: Electron transport through conduction channel in a thin film and 1-D nanostructures upon molecular adsorption
Figure 1.2: Schematic diagram of electrical detection of biosensors: A.) chemiresistor B.) backgated chemical field effect transistor (FET), and C.) ion gated field effect transistor (IGFET)
Figure 1.3: Effect of debye screening length with respect to the carrier concentration of various semiconductors. The red region represents the tunable carrier concentration whereas the blue region signifies the diameter range of 1-D nanostructures able to synthesize to date. The intercept between red and blue regions lying underneath the black line indicates the potential to fully deplete/accumulate the sensors by an electrostatic gating effect.
Table 1.1: Summary of properties of materials can potentially be employed for bioFETs

<table>
<thead>
<tr>
<th>Material</th>
<th>ZnO</th>
<th>In$_2$O$_3$</th>
<th>SnO$_2$</th>
<th>Si</th>
<th>SWNT</th>
<th>PPy</th>
<th>PANI</th>
<th>PEDOT</th>
<th>InSb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier Type</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P,N</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>$E_g$ (eV)</td>
<td>3.36</td>
<td>3.75</td>
<td>3.6</td>
<td>1.1</td>
<td>0.01-0.45</td>
<td>3.16 (intrinsic)</td>
<td>3.94 (intrinsic)</td>
<td>1.6</td>
<td>0.18</td>
</tr>
<tr>
<td>Dielectric constant</td>
<td>8.5</td>
<td>8.9</td>
<td>13.5</td>
<td>11.7</td>
<td>10</td>
<td>250</td>
<td>260.87</td>
<td>3.1</td>
<td>18.7</td>
</tr>
<tr>
<td>Carrier Density (cm$^3$/V.s)</td>
<td>1E16-18</td>
<td>6.6E18-1E19</td>
<td>1E17-6E20</td>
<td>1E15-21</td>
<td>1.6E19</td>
<td>1E16-23</td>
<td>1E18</td>
<td>1E16-20</td>
<td>1.7E16</td>
</tr>
<tr>
<td>Carrier Mobility (cm$^2$/V.s)</td>
<td>60-205</td>
<td>90-110</td>
<td>10-40</td>
<td>500</td>
<td>1E-5</td>
<td>1E-5</td>
<td>0.4-2.11</td>
<td>80.000</td>
<td></td>
</tr>
<tr>
<td>FET Carrier Density (cm$^3$/V.s)</td>
<td>5.2±2.5E17</td>
<td>2.3E7</td>
<td>1.5E8</td>
<td>1E18-19</td>
<td>9E6</td>
<td>1.9-2.6E21</td>
<td>6.2E18</td>
<td>1.6E18</td>
<td></td>
</tr>
<tr>
<td>FET Carrier mobility (cm$^2$/V.s)</td>
<td>13±5</td>
<td>98.1</td>
<td>40</td>
<td>30</td>
<td>79000-220</td>
<td>0.56-1.7</td>
<td>0.8</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>$p_I$</td>
<td>9.5</td>
<td>8.7</td>
<td>4.1</td>
<td>3.2 (SiO$_2$)</td>
<td>3.5 (p-SWNT)</td>
<td>1.2 (COOH-SWNT)</td>
<td>9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimension</td>
<td>(D) 50-200nm (NT) 10nm</td>
<td>(D) 10-30nm</td>
<td>(W) 30-300nm (T) 10-30nm</td>
<td>(D) 5-30nm</td>
<td>(D) 1-3nm</td>
<td>(D) 45-320nm</td>
<td>(D) 200-250nm, 30-40nm wall thickness</td>
<td>(D) 20-120nm</td>
<td>(D) 35-150nm</td>
</tr>
<tr>
<td>Hydrophobicity/ Hydrophilicity</td>
<td>Hydrophilic</td>
<td>Hydrophilic</td>
<td>Hydrophilic</td>
<td>Hydrophilic</td>
<td>Intrinsically hydrophobic, but varied depending on functional group</td>
<td>Intrinsically hydrophobic, but varied depending on dopants</td>
<td>Intrinsically hydrophobic, but varied depending on dopants</td>
<td>Intrinsically hydrophobic, but varied depending on dopants</td>
<td>Unstable</td>
</tr>
<tr>
<td>Stability</td>
<td>Unstable in O$_2$, need molecular passivation</td>
<td>Unstable in O$_2$, need molecular passivation</td>
<td>Unstable in O$_2$, need molecular passivation</td>
<td>Stable</td>
<td>Stable</td>
<td>Dopant dependent stability</td>
<td>Unstable at pH 7.4</td>
<td>Dopant dependent stability</td>
<td>Unstable</td>
</tr>
</tbody>
</table>
Table 1.2: Summary of different bioreceptors employed in chemiresistive/chemFET biosensors

<table>
<thead>
<tr>
<th>Affinitive binders</th>
<th>Antibody</th>
<th>scFvs</th>
<th>Aptamers</th>
<th>DNA</th>
<th>PNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>~10nm</td>
<td>~3nm</td>
<td>~2nm</td>
<td>0.34nm/bp</td>
<td>0.34nm/unit</td>
</tr>
<tr>
<td>Affinity</td>
<td>Low nM-pM</td>
<td>Low nM-pM</td>
<td>Low nM-pM</td>
<td>Low nM-pM</td>
<td>Low nM-pM</td>
</tr>
<tr>
<td>Specificity</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Production</td>
<td>In vivo biological system</td>
<td>In vitro chemical process</td>
<td>In vitro chemical process</td>
<td>In vitro chemical process</td>
<td>In vitro chemical process</td>
</tr>
<tr>
<td>Target range</td>
<td>Immunogenic compounds</td>
<td>Immunogenic compounds</td>
<td>Ions, small organic molecules, protein, whole cells, etc.</td>
<td>DNA, RNA</td>
<td>DNA, RNA</td>
</tr>
<tr>
<td>Batch to batch variation</td>
<td>Significant</td>
<td>Little or no</td>
<td>Little or no</td>
<td>Little or no</td>
<td>Little or no</td>
</tr>
<tr>
<td>Thermal denaturation</td>
<td>Irreversible</td>
<td>Irreversible</td>
<td>Reversible</td>
<td>Reversible</td>
<td>Reversible</td>
</tr>
<tr>
<td>Shelf-life</td>
<td>Limited</td>
<td>Limited</td>
<td></td>
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</tbody>
</table>
CHAPTER 2

WAFFER-SCALE FABRICATION OF SINGLE POLYPYRROLE NANORIBBON BASED AMMONIA SENSOR

Abstract

Ultra long (> 1 cm) polypyrrole (PPy) nanoribbons were batch-synthesized to pre-determined locations on 4 inch Si wafers and flexible polyimide film with controlled thicknesses and widths via modified Lithographically Patterned Nanowire Electrodeposition (LPNE), which combines top-down “photolithography” and bottom-up “electrodeposition”. The excessive lengths and lithographic approach enable subsequent patterning of electrodes for direct device integration. Electrical transport properties were investigated by temperature dependent measurements, and back-gated FET measurements were performed to study the properties with respect to the conductivity of PPy nanoribbons. To demonstrate the utility of this approach these nanoribbon devices were interrogated as gas sensors in a conductometric fashion. They displayed excellent sensitivity toward ammonia vapor with a detection limit of in the sub ppm. The sensitivity toward ammonia vapor was tuned by the conductivity of the PPy nanoribbons via chemical de/doping.
2.1 Introduction

One-dimensional nanostructures, such as nanowires, nanotubes, and nanobelts, are attractive platforms for sensors as their high surface-to-volume ratio has the potential to enhance responses to surface processes, and diminutive sizes endow high density systems characteristic of complex olfactory organs. Among the myriad of actively studied nanomaterials, conducting polymers, such as polyaniline (PANI) and polypyrrole (PPy), offer an intriguing combination of properties, with electrical behavior tunable from insulator to near metallic and polymeric mechanical characteristics providing flexibility and low density. In addition, their room temperature operation, diverse monomer chemistry, and ease of functionalization have prompted the use of conducting polymers in chemical and biological sensors with various transduction mechanisms including conductometric, electrochemical, and field effect transistors (FET).

Various synthesis techniques, from physical to chemical and electrochemical deposition, utilizing both soft and hard templates, have been successfully reported, along with less traditional approaches such as electrospinning, electron-beam (E-beam) lithography, and dip-pen lithography, to fabricate conducting polymer nanowires with precisely controlled dimensions. The major challenge for most of these fabrication techniques is accurate positioning of the polymeric constructs for reproducible integration with existing micro-/macro fabrication. While some methods have addressed these issues, they are typically associated with costly and time consuming serial patterning techniques, such as atomic force microscopy (AFM), focused ion beam (FIB), and e-beam lithography. Moreover, practical and fundamental constraints of each approach impose limits to the lengths and aspect ratio that have been demonstrated with each process.
In this work, we employ Lithographically Patterned Nanowire Electrodeposition (LPNE) for the first time to fabricate ultra-long (> 1cm) complex shaped PPy nanoribbons. LPNE overcomes the drawbacks encountered with other synthesis techniques by combining attributes of top-down “photolithography” and bottom-up “electrodeposition” approaches to fabricate nanowires to pre-determined locations with precisely controlled widths and thickness.\textsuperscript{19,20} The incredible lengths and precise placement of these nanoribbons facilitate alignment of numerous lithographically patterned electrode along the length of PPy nanoribbons anchoring the wires to the substrate and delineating multiple devices from a single wire, permitting four-point probe measurements for reduction of contact resistance. Furthermore, LPNE is a cost-effective process with a wafer-scale batch nature and reproducibility that is amenable to manufacturing. PPy nanoribbons with patterned electrodes fabricated using LPNE demonstrated significant potential as chemical gas sensors based on chemiresistive responses to ammonia vapor. Finally, the sensitivity toward ammonia was controlled by tuning the conductivity of PPy nanoribbons.

### 2.2 Experimental procedure

Commercial p-type silicon wafers with thicknesses of 500-550 microns and a thermally deposited oxide layer of 300 nm (Ultrasil Corporation) were used as substrates. Nickel, which served as the sacrificial layer, was e-beam evaporated at a rate of 1.0 Å/s by a Tamescal BJD-1800 e-beam evaporator to a thickness of 100 and 25 nm. The Ni layer was then spin coated with an adhesion promoter (ShinEtsuMicroSi Microelectronic Material, Primer P20) at 1000 r.p.m for 2 s followed by 4000 r.p.m. for 30 s to enhance adhesion between Ni and the subsequent photoresist. A positive
photoresist layer (Rohm and HAAS Electronic Material, S1813) was then coated at the same conditions as the adhesion promoter coating and transferred to a hot plate at 110°C for 5 min. After soft baking, the pattern was made by photolithographically exposing the wafer to ultraviolet lamp at wavelength of 365 nm and intensity of 5 mW/cm² for 7 s. The pattern was then developed by immersing the wafer into an aqueous developer (Rohm and HAAS Electronic Material, 351) solution diluted at a ratio of 1 to 5 for 50 second, rinsing with pure water, and blow drying with high purity N₂.

Commercial Ni etchant (TFB Transene Company, Inc.) was used to remove the exposed Ni until it was etched completely to the edge as shown in Figure 1. After chemical etching, electrochemical etching technique was exploited to produce Ni nanoband after it was electrochemically recessed. The electrolyte for this anodic dissolution was 0.1M Potassium Chloride (KCl) and 24 mM Hydrochloric acid (HCl). The potential was applied at 0.02V versus SCE (Saturate Calomel Electrode) and Pt-coated Ti sheet as counter electrode for 5 min. These reference electrode and counter electrode were used for all electrochemical experiments. The dissolution of Ni was achieved in one compartment with three electrode cell. After formation of Ni nanobands, the wafer was rinsed with nanopure water. Ni nanobands were used as electrodes for electropolymerization of PPy nanoribbons. Lithium Perchlorate (LiClO₄) (0.2M, Fisher Scientific) solution was made and purged with ultra-high purity N₂ gas for an hour. Pyrrole monomer from Sigma Aldrich was purified by distillation before use, and 0.5M distilled Pyrrole was add into purged LiClO₄ solution. The mixture solution of LiClO₄ and Pyrrole was mixed about 15 minutes prior to use. Electropolymerization of PPy nanoribbons was carried out in a single 1000 ml compartment, three electrode cell at an
applied potential of 0.7V vs SCE. The width of the PPy nanoribbons was varied by controlling the duration of deposition.

After synthesis of PPy nanoribbons, the wafer was submerged in Acetone to remove the positive photoresist. Lift-off photolithography was used to pattern electrode onto nanoribbons. The wafer was then spin coated with adhesion promoter (P20) at the rotation rate of 1000 rpm for 2 s and 3000 rpm for 30 s. The positive photoresist (AZ Microelectronic Materials, 5214E) was dropped onto wafer until it fully covered the whole surface area of the wafer, and the wafer was rotated again at the 1000 rpm for 2 s and 3000 rpm for 30 s. The wafer was soft baked at 110°C for 5 min and exposed at wavelength of 365 nm and intensity of 5 mW/cm² for 7 s. The wafer was soaked with the solution of developer (AZ Electronic Materials, 400K) and water in the ratio of 1 to 4 and dried with ultra-high purity N₂.

Chromium (adhesion layer between gold and SiO₂ surface) and gold with thickness of 20 nm and 180 nm were deposited onto the wafer by e-beam evaporator at rate of 1.0 Å/s and 3.0 Å/s, respectively. The wafer was then soaked with acetone until the gold electrode patterns were developed. Ultra high purity N₂ was then gently sprayed to dry the surface of the wafer. Nickel was removed after patterning electrode by immersing wafer in 2% HNO₃. An optical microscope, scanning electron microscopy (SEM), and atomic force microscopy (AFM) were used to characterize PPy nanoribbons.

Temperature coefficient of resistance of PPy nanoribbons were achieved by measuring temperature I-V scanning from potential of -1 to 1 V. Carrier mobility was obtained from transfer characteristics by sweeping gate potential from -10V to 10V with fixed source-drain voltage of 2V. The conductivity-dependent field effect mobility was characterized by chemically doping with 1M Perchloric acid (HClO₄) and overoxidizing
with 1M NaOH. After material characterization, PPy nanoribbons configured as chemiresistor was implemented for study of gas sensing performance. The constant potential of 0.5V was applied during sensing experiment. After exposing sensor devices to carrier gas (dry air), baseline of sensors was established. PPy nanoribbons were then tested with ammonia vapor at various concentrations from 500 ppb\textsubscript{v} to 50 ppm\textsubscript{v}. The total flow rate is kept constant at 200 sccm for entire sensing experiment.

2.3 Result and discussion

The modified LPNE process is illustrated in Figure 2.1, starting with an e-beam deposited sacrificial layer (green), followed by spin coating of a positive photoresist PR (purple). Patterning the substrate is realized by exposing the wafer to UV light under a mask and developing the pattern by soaking the wafer in the developer solution. Undercutting of the sacrificial layer in the step C is produced first by chemically etching the exposed Ni film to yield a nanoband flush with the PR, and subsequent electrochemical etching to create the trench. The nanoband serves as a working electrode for electropolymerization of PPy nanoribbons, followed by removal of the photoresist. Finally, lift-off photolithography was used to pattern Au electrodes onto PPy nanoribbons, and the remaining sacrificial layer was dissolved in an acidic solution (step E, F).

Several distinct features were unique to our process for synthesis of conducting polymer nanoribbons by LPNE. The first is the two-step process adopted to ensure complete removal of the exposed Ni film by chemical means with smooth undercut profiles produced electrochemically. Although the chemical etch step did impose a slight
recess on the Ni nanoband, the trench formation was very uneven and relatively insensitive to time, with nm to <1μm undercuts after 5 min. The first step was necessary to prevent island formation due to the large open areas typical of our custom patterns. While the islands were not completely detrimental to our process they did lead to irregularities during electrode patterning and were deemed undesirable. The formation of various trench depths was achieved by controlling the anodic dissolution duration, with a nearly constant etch rate displayed for both 25 and 100 nm thick sacrificial layers for all time periods < 10 min as illustrated in Figure 2.2.

After formation of the trench, Ni nanobands were used as working electrodes for electropolymerization of the PPy nanoribbons by applying an oxidative potential in the presence of the pyrrole electrolyte. In many systems, electropolymerization of conducting polymers on oxidizable metals has posed a challenge. Preventing dissolution of the Ni sacrificial layer in our case was overcome with a highly concentrated monomer bath of 0.5M pyrrole and 0.2M LiClO₄ and the relatively low polymerization potential of pyrrole, 0.7 V vs SCE. This solution was made and purged with ultra-high purity N₂ (99.999%) gas to prevent chemical oxidation of the pyrrole by dissolved oxygen. Electropolymerization of PPy nanoribbons was performed anodically in a three electrode configuration. The width of the PPy nanoribbons was controlled by regulating the deposition time as shown in the growth rate plot of PPy nanoribbons in Figure 2.3 for width down to 300nm. Optical and SEM images in Figure 2.4A-D illustrate wafer-scale fabrication of PPy nanoribbons to pre-determined locations with enlargement of the different nanoribbon patterns on silicon wafers and a flexible polyimide film (Figure 2.4B and 2.5). Figure 2.4 also exemplifies how LPNE can be utilized to fabricate PPy nanoribbons exceeding lengths of 1 cm, as PPy is electrodeposited along the entire
electroactive nanoband perimeter forming one continuous PPy loop. AFM analysis in Figure 2.6 shows the height of polypyrrole nanoribbons (e.g. 25 nm) nearly matches the thickness of the sacrificial nickel layer with an average thickness of 23.74 nm. The AFM image also depicts the granular morphology of the nanoribbon with an average grain size of 0.0293 µm² mimicking that of the SiO₂ with an average grain size of 0.0290 µm², suggesting means to manipulate the ribbon morphology.

Translating these centimeter long PPy nanoribbons into addressable devices requires additional steps to electrically contact and physically bind these as-synthesized structures. The novelty of such lengths is the ease of integrating tens or hundreds of lithographically patterned electrodes, but the drawback to LPNE is the limited adhesion between the nanoribbons and substrate, since the PPy is electrodeposited only on the Ni thin film sidewall. Consequently, once the sacrificial layer was completely removed the nanoribbons were readily disrupted. The protocol was accordingly modified to incorporate lithographically patterned electrodes prior to complete removal of the sacrificial Ni to provide electrical contact and solid mechanical joints. These contacts are more robust than previous Ni sidewall contacts lithographically patterned from the sacrificial layer, enabling characterization of nearly mm long PPy segments.

The average room temperature conductivity of as-synthesized PPy nanoribbons in ambient pressure and lighting was 15 ± 1.29 S/cm. These values are consistent with the range of conductivities reported for one dimensional PPy nanostructures,8,21 which can span over six orders of magnitude (160-0.0001 S/cm) with the highest conductivities reported for FIB contacted PPy nanowires and the most insulating form observed for segmented PPy nanowires, probably due to a reducing current during electrodeposition of subsequent metallic segments and strong basic conditions to remove the
These discrepancies are anticipated as the synthesis conditions (temperature, solvent, dopant type and concentration, applied potential, etc.) are known to strongly affect the polymer conformation and dopant loading, factors that dictate the conductivity and transport behavior. In contrast to the conductivity variability of several fold along a single electrochemically template-synthesized PPy nanowire, LPNE fabricated nanowires displayed surprising uniformity with a standard deviation of only 1.29 S/cm, less than ten percent. Figure 2.7A contains the I-V response of a single PPy nanoribbon as a function of temperature, characterized after a month of storage in ambient conditions. The current-voltage plot showed ohmic contact between the nanoribbon and electrodes for a voltage range of -1V to 1V. The conductivity of this mm long PPy nanoribbon, displayed in the inset, at room temperature was calculated to be 5.96 S/cm, indicating aging/oxidation of the polymer, but still comparable with PPy nanowires fabricated by others.

Nonetheless the conductivity alone is not sufficient to characterize these materials. Electron transport properties of conducting polymers are unique, in that, unlike inorganic materials that have both spherical building blocks, they are composed of 1-dimensional chains that have both polydispersity and packing arrangement or degree of disorder in addition to doping level. As a result, carrier transport occurs by both intra- and interchain procession. Although the individual polymer chains may be highly conductive, the transport behavior is dominated by interchain transport, which is generally described by a hopping or tunneling mechanism for low and high conductivity/disorder, respectively. The appropriate transport model for conducting polymer materials can be characterized by temperature-dependent charge transport. In this study, three samples were analyzed by temperature dependent studies; one 3 µm
wide sample with a conductivity of 6.5 S/cm and two 500 nm wide samples with conductivities of 1.25 S/cm and 0.003 S/cm. These particular devices were chosen to contrast both nanowire width and conductivity. The negative temperature coefficient of resistance (TCR), described as $R'(dR/dT) = \alpha$, signifies semiconducting typical behavior of electrochemically synthesized 1-D PPy nanostructure.\textsuperscript{25} The magnitude of the TCR also denotes the relative disorder, which is relatively small as observed from Figure 2.7B, a typical response for these nanoribbons. Semiconducting behavior was further substantiated by resistance in the high temperature region (100-295K), where the resistance demonstrated an Arrhenius relationship with temperature, displayed in Figure 2.7B, with an activation energy of 60 meV, which is comparable to that reported by Mirkin's group.\textsuperscript{23} However, for disordered materials with localized carriers, such as conducting polymers, a more insightful parameter for the metal insulator transition is the reduced activation energy, $W = (d[ln(\sigma)]/d[ln(T)])$,\textsuperscript{25} producing a positive slope for metallic transport, a negative slope for insulating behavior, and a zero slope in the critical regime. In Figure 2.8, the relatively weak temperature dependent changes in the $W$ value for the thicker sample suggest the ribbon is approaching the critical regime. On the other hand, the two 0.5 µm wide samples exhibited positive slopes of 0.60 and 0.61 for the more and less conductive sample, respectively, above 100K, demonstrating relatively less disorder than the wider sample. Although the $W$ value is dependent on the dopant concentration, the slope has been previously shown to be independent of dopant level in conducting polymer nanowires, supporting rational for use of $W$ in characterizing the disorder of the deposited conducting polymer.\textsuperscript{26} The positive slopes observed in the thinner ribbons is also consistent with thin films studies demonstrating more crystalline and ordered phases during the earlier stages of electrodeposition.\textsuperscript{27}
This is a feature unique to LPNE patterned PPy nanoribbons not possible for solid electrodeposited PPy nanowires in nanoporous templates due to the shift in the growth direction from parallel to perpendicular to the wire axis for potentially more crystalline nanowires.

Since the temperature dependent studies indicate semiconducting behavior of the PPy nanoribbons, back-gated FET measurements were performed to further elucidate charge transport characteristics of these nanoribbons. A three terminal configuration was used as shown in the inset of Figure 2.9A, where the gate potential was applied through a highly doped silicon wafer with a SiO$_2$ thickness of 300 nm from -10V to 10V at a fixed $V_{SD}$ of 2V. As shown in Figure 2.9A, these PPy nanoribbons behave as p-type semiconductors and their FET parameters e.g. field effect carrier mobility (Figure 2.9B) were dependent upon the conductivity (about 0.003 to 10 S/cm), which was controlled by soaking the PPy nanoribbons in concentrated HClO$_4$ and NaOH solution. The charge transferred slower in NaOH-treated PPy nanoribbons than in HClO$_4$-doped PPy nanoribbons, presumable due to dedoping that occurs when exposing PPy nanoribbons to a basic solution as opposed to increase in doping level in HClO$_4$. Although on-off ratios were poor compared to previously reported conducting polymer back gated devices, mobilities of nearly 4 cm$^2$/Vs were observed after doping, which is an order of magnitude greater than previously reported PPy nanowire FET devices and two orders greater than thin film devices (Figure 2.9C). Ultimately, these FET results indicate that conductivity is dependent on carrier mobility and carrier concentration. The overall variation in transport behavior of these PPy nanoribbons provides an intriguing scenario for correlation with sensing performance, which, to the best of our knowledge, has not been previously pursued. The response of single PPy nanoribbons with different
conductivities and thicknesses towards ammonia vapor were investigated to illuminate contributions from dopant concentration and disorder, respectively. Ammonia is a widespread compound of interest commonly found in industrial refrigeration systems and the production of fertilizers and explosives. Ammonia is also a component in vehicle emissions resulting from rich air-fuel conditions and catalytic converter malfunction contributing to the formation of fine particulate matter (PM$_{2.5}$), which could be mitigated or better controlled with a sensor feedback system. Lastly, elevated concentrations of ammonia in exhaled breath, 50-100 ppb, can be used as a noninvasive diagnosis of renal disorders or ulcers. The sensing mechanism for NH$_3$ with PPy is a well-studied interaction, whereby ammonia donates an electron to the polymer backbone to produce an ammonium ion that reduces a bi/polaron constituent on the chain by inducing charge neutrality with the dopant. The overall effect of ammonia binding to the PPy is a reduction in charge carriers or mobility and a rise in resistance. These devices were operated in a chemiresistive configuration by monitoring the change in resistance (Figure 2.10A,B). Dry air was selected as the carrier gas and the exposure to ammonia, shown on a logarithmic scale (Figure 2.10A,B), was conducted between concentrations of 500 ppb, to 50 ppm$_v$. The flow rate was held constant at 200 sccm for the duration of the experiment by means of mass flow controllers. Rapid and complete recovery at low NH$_3$ concentrations was observed, however, higher concentrations ($\geq$ 5 ppm$_v$) generated sluggish recoveries that did not return to the baseline even after several hours. These hysteretic responses may be due to dopant pumping or conformational changes in the polymer chain and is indicative of a bulk nanowire response. This indicates the sensitivity of ammonia gas sensor can be improved by controlling the conductivity of PPy nanoribbons (Figure 2.10C). The sensing performance of PPy nanoribbon based
ammonia gas sensor is strongly influenced by the debye length ($\lambda_D$) or charge interaction zone which is affected by charges, temperature, dielectric constant and carrier concentration of materials. Thus, carrier concentration of PPy nanoribbons, which is tailored by manipulation of conductivity via doping/dedoping process, can impact the charge depletion/accumulation region ($\lambda_D$). Smaller numbers of carrier concentration observed in PPy nanoribbon with less conductivity produce significant effect within conduction channel of PPy nanoribbons. Despite of slower charge transfer in less conductive region of PPy nanoribbon, the sensitivity towards ammonia vapor is improved by 80% at 1 ppm due to smaller conduction channel at the interaction zone that permits charge carriers to flow. Within the wider scope of this paper, these results provide evidence for carrier mobility and carrier concentration dependent sensitivity. While dopant levels, described as the work function, have been previously demonstrated to tune the selectivity of conducting polymer gas sensors to organic vapors, this is the first demonstration of enhanced sensitivity to an electron donator/acceptor with increased reduction of a conducting polymer.30,32,33

2.4 Conclusion

LPNE was used to fabricate one dimensional conducting polymer nanostructures with integrated microelectrodes for sensing applications. The fabrication technique combines attributes of photolithography and electrodeposition into one step, synthesizing high aspect ratio conducting polymer nanoribbons to precise locations on silicon/silicon dioxide substrates with controlled dimensions. The process permits these cm long nanoribbons to be readily interfaced with existing microfabrication technologies, which distinguish individual devices by their placement, offering a manufacturable
method to fabricate nanostructured conducting polymers. Temperature dependent electrical properties of the PPy nanoribbons exhibited semiconductor properties based on the negative TCR and activation energies. These nanoribbons were also shown to make excellent gas sensors with detection down to sub-ppm concentrations of NH$_3$ at room temperature. The sensitivity toward ammonia gas was tuned by chemically modulating the conductivity of PPy nanoribbons.
2.5 References


(2) Cui, Y.; Wei, Q. Q.; Park, H. K.; Lieber, C. M. Science 2001, 293, 1289-1292.


Figure 2.1: Schematic illustration of modified LPNE process. Sacrificial layer Ni (green) is e-beam deposited onto substrates, followed by spin coating of Photoresist (PR) in A. Patterned by exposing the spin coated sample to UV light, followed by dissolving exposed PR by developer solution in B. Removal of exposed Ni by chemical etching, followed by electrochemical etching to produce Ni nanobands as substrate for electropolymerization of PPy nanoribbons in C. Electropolymerization of PPy nanoribbons is carried out at Ni nanobands in D. Removal of PR and integration of gold electrode by lift-off photolithography technique, followed by removal of Ni in E and F.
Figure 2.2: The trench depth was a function of etching time for 25 nm and 100 nm thick nickel
Figure 2.3: The width of polypyrrole nanoribbons as a function of deposition time.
Figure 2.4: Optical images of PPy nanoribbons on 4” silicon substrate (A) and polyimide film (B). Optical images which magnify the patterned PPy nanoribbons with integration of gold electrodes. (D) SEM image of single PPy nanoribbons integrated with gold electrodes.
Figure 2.5: Optical images of different patterned polypyrrole nanoribbons with integrated gold electrodes fabricated on polyimide films via Lithographically Patterned Nanowire Electrodeposition (LPNE).
Figure 2.6: Atomic Force Micrograph of PPy nanoribbons with 25 nm thickness. Analysis of histogram peak shows the step height of 23.74 nm, comparable to height of sacrificial Ni layer.
Figure 2.7: (A) I-V characteristics of PPy nanoribbon shown in inset with various temperature where A = 295K, B = 250K, C = 210K, D = 170K, E = 130K, and F = 50K, (B) Plot of temperature coefficient of resistance ($\alpha$) depicts semiconducting behavior of PPy nanoribbons and inset shows the Arrhenius plot which activation energy near room temperature of 60 meV.
Figure 2.8: The reduced activation energy (W), $d[\ln(\sigma)]/d[\ln(T)]$, as a function of temperature.
Figure 2.9: $I_{ds}$-$V_g$ Characteristic of PPy nanoribbons (A) and Back gated FET properties as a function of conductivity of PPy nanoribbons: carrier mobility (B)
Figure 2.10: PPy nanoribbons based gas sensor toward different concentration of ammonia vapor at conductivity of 6.5 S/cm (A) and 0.003 S/cm (B). The black and red lines indicate the gas sensing response and duration of exposure of ammonia vapor. Effect of sensitivity of \( \text{NH}_3 \) sensing on conductivity of PPy nanoribbons (C).
CHAPTER 3

VIRAL PLANT PATHOGENS DETECTION USING SINGLE POLYPYRROLE (PPy) NANORIBBON IMMUNOSENSOR

Abstract

Single polypyrrole (PPy) nanoribbon based chemiresistive immunosensors were massively batch-fabricated using lithographically patterned nanowire electrodeposited polypyrrole nanoribbon in the pre-determined locations followed by surface covalent immobilization of the capture polyclonal antibodies via N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide(EDC)/N-hydrosuccinimide (NHS) chemistry. Excellent sensing performance of the device was demonstrated by detecting cucumber mosaic virus (CMV) using CMV polyclonal antibodies functionalized PPy nanoribbon. The sensitivity of the nanoribbon immunosensors was enhanced by reducing the thickness of the PPy nanoribbon from 100 nm to 25 nm and improving semiconducting properties (i.e., reducing the conductivity of the PPy nanoribbon). Although the sensitivity of the devices was improved by reducing the ionic strength of the pH buffer solutions (i.e., 10 mM PBS to 10 mM PB), the reliability and reproducibility of the sensors were dramatically reduced. At the optimum condition, the sensors showed excellent sensitivity with low and upper detection limit of 10 ng/ml and 100 µg/ml, respectively.
3.1 Introduction

Cucumber mosaic virus (CMV), an economically important virus belonging to the genus Cucumovirus (family Bromoviridae), is commonly found throughout the world and is transmitted by different aphid species to a wide range of vegetable crops including cucumbers, tomatoes, grapes, peanut, tobacco, etc.\textsuperscript{78} Similar to other plant viruses, no chemical substances have been reported to significantly prevent and eliminate CMV infection in plants. Current CMV mitigation strategies mainly involve eradication of infected sources so that they will not serve as inocula for other infected agricultural crops in the field.\textsuperscript{79} Thus, early detection is of the utmost importance and key to the successful management of CMV.

Many serological and molecular detection techniques have been developed and exploited to identify and quantify viral plant pathogens ranging from enzyme-linked immunosorbent assays (ELISA), including direct double antibody sandwich ELISA (DAS-ELISA) and triple antibody sandwich ELISA (TAS-ELISA), electrochemical immunoassay (ECIA) to real-time PCR.\textsuperscript{79-81}

Although these techniques can effectively detect viral plant pathogens, they are bound to laboratory settings, requiring well-trained individuals, costly instruments, and time-consuming processes. Development of rapid, accurate, reliable, and miniaturized field deployable sensors for minimally trained personnel has prompted research on one-dimensional (1-D) semiconducting nanostructures based chemiresistors or chemical field effect transistors (ChemFET). Owing to their ultra-high surface area to volume ratio, enhancing surface adsorptive capacity, and tunable electrical properties, these label-
free, ultra-sensitive, and selective electronic biological/chemical sensors show a great promise towards fulfilling this need. When a molecule binds on the surface of a nanostructured transducer (e.g., PPy nanoribbon), such a minute perturbation leads to depletion/accumulation of charge carriers within the bulk of the nanostructure which results in significant alterations of their electrical properties. This feature greatly improves the sensitivity as well as the low detection limit. One-dimensional nanostructures including single-walled carbon nanotubes (SWNTs), metal oxide nanowires (NWs), and silicon NWs configured as chemiresistors and ChemFETs have been developed for the detection of various biomolecules such as viruses, DNA, proteins, bacteria and cells.\textsuperscript{21,23,24,30,69,73,82} While these works demonstrated greater potential for advancing the sensing technology, one of the major challenges is the development of scalable, reproducible and cost effective methods to fabricate and assemble high density devices.

Despite their short history compared to inorganic materials, conducting polymers such as polypyrrole (PPy), polyaniline (PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT) have emerged as excellent transducers for label-free electronic chemical/biosensors.\textsuperscript{22,27,83,84} Besides the aforementioned advantages of 1-D nanostructures, 1-D conducting polymer nanostructures can be chemically or electrochemically synthesized under mild conditions, which allow entrapment of biorecognition molecules during electropolymerization, bypassing post-surface immobilization treatment.\textsuperscript{1,42,85} Their diverse monomer chemistries and derivatives also promote post-surface covalent immobilization using well-studied bioconjugation chemistry.\textsuperscript{86} Herein, we demonstrate the detection of CMV using anti-CMV polyclonal antibodies functionalized PPy nanoribbon-based chemiresistive immunosensors. The
lithographically patterned nanowire electrodeposition (LPNE) technique previously developed by our group was employed to massively batch-fabricate PPy nanoribbons at pre-determined locations with integration of patterned microelectrodes.\textsuperscript{60} \(N\)-(3-dimethylaminopropyl)-\(N'\)-ethylcarbodiimide (EDC)/\(N\)-hydrosuccinimide (NHS) chemistry was used to surface functionalize anti-CMV onto the PPy nanoribbons. The sensing performance of anti-CMV functionalized PPy nanoribbon-based immunosensors was optimized by adjusting dimensions, electrical conductivity, and the ionic strength of pH buffer.

3.2 Material and methods

3.2.1 Device fabrication and assembly

Single PPy nanoribbons with dimension of 500 nm in width and thicknesses ranging from 25-100 nm were batch-synthesized with integrated gold microelectrodes using LPNE technique as illustrated in Figure 3.1A. The detailed fabrication method is reported in our prior work.\textsuperscript{60} First, a sacrificial Ni layer with thicknesses ranging from 25 nm to 100 nm was e-beam evaporated onto a 4” thermally oxidized p-type Si wafer (Ultrasil Corporation) with SiO\(_2\) thickness of 300nm in step (1). As reported in our earlier work, the thickness of a sacrificial Ni layer will dictate the thickness of nanoribbons.\textsuperscript{60,87} Sequential spin coating of adhesion promoter P20 (ShinEtsuMicroSi Microelectronic Material, Primer P20) and photoresist (PR) 5214E (AZ Microelectronic Materials, 5214E) were performed at 1000 r.p.m. for 2s and 3000 r.p.m. for 30s, respectively. The substrates were baked at 110°C on a hotplate for 5 min prior to photolithographically defining a pattern. A pattern was photolithographically defined by exposing the PR coated substrate to an ultraviolet lamp (applied wavelength of 365 nm with intensity of 5
mW/cm²) for 7s, followed by immersing in a diluted develop solution (AZ Electronic Materials, 400K) to develop the pattern. The patterned substrates were dried with ultra-high purity N₂ in step (2). Exposed Ni film was then chemically dissolved by selective Ni etchant (TFB Transene Company, Inc.), followed by electrochemical etching of Ni at an applied potential of 0.02V vs. saturated calomel electrode (SCE) in 0.1 M KCl + 24 mM HCl to form a Ni nanoband. Finally, PPy nanoribbons were electrochemically synthesized at the Ni nanoband in step (4).

After PPy nanoribbon synthesis, the PR layer was removed by soaking the substrate in an acetone bath for 5 min. Lift-off photolithography was used to integrate microelectrodes onto nanoribbons. The same protocol for spin coating of PR and patterned development described earlier was exploited. Ti as an adhesion layer and gold as an electrode material with thicknesses of 20 nm and 180 nm were sequentially e-beam evaporated. The substrate was submerged in an acetone bath to attain patterned microelectrodes in step (5). Excess Ni was then selectively removed by 2%v/v HNO₃ solution, and the samples were dried with high purity N₂ gas in step (6).

3.2.2 Biofunctionalization of PPy nanoribbons with capture CMV antibodies and sensing measurement

As shown in Figure 3.1B, our method allows fabricating 16 individually addressable single PPy nanoribbons with 3 µm gap microelectrodes at the center of the sample. The sample was then mounted on a homemade Teflon incubation cell to enclose the area that the PPy nanoribbons were located and improve fluid handling during biofunctionalization and sensing experiment. Electrical measurement was carried out by probing source (S) and drain (D) electrodes using SOIC test clips (Allied
Electronics, Texas) connected to integrated Keithley switching and system sourcemeter 2636A (Keithley Instrument, Ohio). The current-voltage (I-V) responses of the PPy nanoribbons were obtained by sweeping applied voltage from -0.5 to 0.5V. The resistance of the PPy nanoribbon was determined by inverting the slope of the I-V responses. Subsequently, polyclonal anti-CMV IgG (Agdia, IN, USA) was immobilized onto the surface of PPy nanoribbon by N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide (EDC) / N-hydrosuccinimide (NHS) chemistry, generating covalent attachment of antibodies on the surface of the PPy nanoribbons.\textsuperscript{22}

In short, the carboxylic acid group of the anti-CMV was activated by EDC/NHS to form an intermediate carboxylate succimidyl ester which then chemically reacted with the active amine group on the surface of the PPy nanoribbon.\textsuperscript{22} To biofunctionalize anti-CMV, the PPy nanoribbons were first incubated in a solution which consisted of 20 µl of 40 mM N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) (Sigma Chemical Co., St. Louis, MO. USA) in 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH = 5.5) and 200 µl of 10 mM N-hydrosuccinimide (NHS) in dimethylsulfoxide (DMSO) (Thermo Fisher Scientific, Rockford, IL, USA). The incubation step was skipped for the unfunctionalized samples, which were later used as controls. After 3h incubation at room temperature, the solution mixture was pipetted out and washed with 10 mM buffer solution containing 0.1% v/v Tween20 and rinsed with buffer solution. The washing and rising protocol was repeated for three times. Both the phosphate buffer saline (PBS) and the phosphate buffer (PB) solutions with the same concentrations (i.e., 10mM) were used, and pH was kept at 7.4. Sensors were incubated with 1 mg/ml Bovine Serum Albumin (BSA) (Sigma-Aldrich Inc., MO, USA) in the buffer solution for 1h at room temperature to prevent non-specific binding. After the incubation, the BSA
solution was drawn and washed with buffer-Tween20 solution followed by the buffer solution. The washing and rinsing step was repeated three times.

Virions of the Fny strain of CMV, CMV-Fny, were purified from infected *Nicotianaclevelandii* plants by the ‘standard’ CMV purification procedure previously described by Ng et al. Purified virions estimated at a concentration of 36 mg/ml by UV absorption at 260nm, assuming an extinction coefficient of 5.0 cm$^2$/mg, were diluted to 1 mg/ml aliquots using PBS solution (pH=7.4). Afterwards, the 1 mg/ml aliquots were used as the experimental stock solutions for further dilutions by PBS and PB solution (pH=7.4) to various CMV concentrations ranging from 10 pg/ml to 100 µg/ml. The resistance of the PPy nanoribbon upon exposure to different concentration of CMV was measured in a buffer solution with CMV after incubation with 150 µl of the CMV solution, and washing and rinsing with the buffer solution.

### 3.3 Results and discussion

Single conducting polymer nanowire based immunosensors demonstrated exceptional sensing performance for detection of proteins and viruses, however a major challenge to synthesize and assemble an individually addressable single conducting polymer nanowire still remained. Although various techniques including magnetic and AC dielectrophoretic alignment were attempted to position conducting polymer nanowires onto the microelectrodes, they are tedious and serial processes for cost effective bath-fabrication of high density sensor arrays. To mitigate this challenge, we exploited LPNE technique to bath-fabricate the PPy nanoribbons with precise placement onto the oxidized Si substrates.
The electrical properties of the PPy nanoribbons were characterized in terms of resistance, which was an inverse slope of I-V responses measured in 150 µl of 10 mM PBS buffer solution (pH = 7.4) between -0.2V to 0.2V. The resistance would be referred to as ‘wet resistance’ henceforth. Figure 3.2A and 3.2B displayed the fabrication and detection scheme of anti-CMV immobilized PPy nanoribbon along with the corresponding I-V characteristics of each step. The wet resistance of the PPy nanoribbons after surface functionalization (step 2) of anti-CMV increased, which may be attributed to change in carrier distribution and conformation within the PPy nanoribbons upon covalent immobilization of the bioreceptors. Physical adsorption of BSA (step 3) to prevent non-specific binding further raised the wet resistances of the PPy nanoribbons, because BSA (pI = 4.7) exhibited overall net negative charges under the working buffer solution. Adsorption of such negatively charged biomolecules on the surface of the p-type semiconducting PPy nanoribbon evidently caused charge depletion at the interaction zone within the PPy nanoribbon conduction channel. Finally, the detection of CMV was realized by a further increase in the wet resistances of the PPy nanoribbons from incubation of 0.1 µg/ml of CMV, because the net negative charges possessed by CMV (pI = 4.7) in buffer solution with a pH of 7.4 induced a charge transfer to the p-type semiconducting PPy nanoribbons.

The sensitivity of the anti-CMV functionalized PPy nanoribbon based CMV immunosensor was evaluated by normalized changes in the wet resistance (∆R/R₀) obtained from voltages range between -0.2 to 0.2V upon incubation of ascending CMV concentrations diluted in 10mM PBS solution between 10pg/ml to 100µg/ml. To verify that the measurable signals were merely from specific antibody-antigen interactions, a control experiment was first performed; the CMV solution was exposed onto the
unfunctionalized PPy nanoribbons. It resulted in a negligible change in the sensitivity ($\Delta R/R_0$) as depicted in Figure 3.3, which indicated that the response to non-specific adsorption was successfully suppressed. After no non-specific binding was validated, the CMV sensing experiment was performed using PPy nanoribbons with initial conductivity ranges of 0.1-1 S/cm. In Figure 3.3, the sensor showed detection toward CMV with a detection limit of 1 µg/ml and the dynamic range spanned up to 100 µg/ml. The detection limit of CMV from a PPy nanoribbon based CMV immunosensor was comparable to that generated by ELISA reported limit of detection as 3µg/ml using monoclonal antibodies as bioreceptors.\(^79\)

It is reported that the charge depletion/accumulation region, also known as the Debye length ($\lambda_D$) caused a change in the conductance/resistance within the conduction channel when perturbations of charged molecules on the surface of 1-D nanostructures occurred. This charge interaction area was intensely affected by the total charge, temperature, materials’ dielectric constant, and carrier density.\(^{25}\) The change in the conductivity of the PPy nanostructures resulted in a carrier density change and greatly influenced the sensing performance. This was supported by our results with PPy nanoribbon based ammonia sensors in which smaller carrier density from the more resistive PPy nanoribbons produced a smaller conduction channel that enabled the flow of charges at the penetration zone.\(^60\) Hence, the sensitivity and detection limit of anti-CMV functionalized PPy nanoribbon based biosensors was also expected to improve if the resistance of the PPy nanoribbon increased. The same experimental sensing protocol using more resistive PPy nanoribbons (0.01-0.1 S/cm) was performed to corroborate the effect of the charge carriers towards CMV detection. As illustrated in
Figure 3.3, the limit of detection shifted to 10 ng/ml and a significant enhancement in $\Delta R/R_0$ was realized at a CMV concentration of 100 ng/ml.

In addition to the Debye screening length within the PPy nanoribbon, the solution Debye length (i.e., solution double layer) should also have a great effect on the electrical biosensor, where it is dependent on the dissolved salt concentration.\textsuperscript{62} Elevated salt concentration drastically decreases the solution Debye length, which may preclude a majority of external charges that influenced 1-D nanostructures. Thus, different buffer solutions (i.e., phosphate buffer saline (PB) and phosphate buffer solutions (PBS)) were examined to determine their effect. Figure 3.4 showed the comparison of the sensitivity towards CMV in 10mM PB and PBS buffer. The conductivity of the nanoribbon was kept in the range of 0.1-1.0 S/cm. Despite greater sample-to-sample variations, the sensitivity and low detection limit of PPy nanoribbons in PB buffer were much greater than PBS buffer, which indicated that a larger solution Debye length allowed greater charge interaction between molecules and PPy nanoribbons.

Another attractive feature of 1-D nanostructures based sensing devices is an ultra-high surface area to volume ratio, which boosts the surface adsorptive capacity. In addition to an enhanced surface area to volume ratio, a wide channel promotes an increased number of immobilized receptors onto the surface and subsequently allows more analytes to bind onto the functionalized receptors. Hence one factor that greatly influenced the sensitivity of biosensors is the thickness or depth of the conduction channel.\textsuperscript{93} For 1-D nanostructures synthesized by the LPNE processes like the PPy nanoribbons, a reduction in size could be achieved by independently controlling the thickness of the sacrificial layer via e-beam evaporation and the width by the duration of electrodeposition. In this study, the width of the PPy nanoribbons remained constant at
500 nm, however their thicknesses varied between 25 nm to 100 nm. Figure 3.5 displayed the thickness dependent sensitivity of PPy nanoribbons based CMV biosensor. The $\Delta R/R_0$ of 0.29±0.05 at a PPy nanoribbon thickness of 100 nm was improved to 0.72±0.24 at a thickness of 25 nm at a CMV concentration of 1 µg/ml with an increase in sensor-to-sensor variation.

Logarithmic dependence of $\Delta R/R_0$ with respect to CMV concentrations was observed. Nair et al stated that the logarithmic relationship can be explained by the diffusion-capture model and Poisson-Boltzmann equation. The kinetics of the biomolecules adsorbed on the surface of the biosensors, the mass transfer of biomolecules set by the concentration gradient at the sensor surface, and the rate of molecular interaction between the target analytes and the bioreceptors was governed by the diffusion-capture model. However, the electrostatic screening by dissolved salt solution hampered modulating the conductance induced by the full charge of the target analytes. Thus, Poisson-Boltzmann equation was employed to account for this effect. The sensitivity of biosensors theorized by this model depicted the logarithmic dependence on both the target biomolecule concentration and the ionic concentration. Thus, not only did the sensitivity decrease by an increase in ionic strength, but the longer incubation time was also expected to achieve the same sensitivity.

3.4 Conclusions

In summary, we have demonstrated applicability of single PPy nanoribbon based chemiresistive immnosensors for CMV detection. The nanoribbon based sensors showed an excellent sensing performance with low detection limit of 1 µg/ml with a wide dynamic range up to 100 µg/ml. Discrete changes in the conductance/resistance of the
PPy nanoribbon upon exposure to CMV resulted from an alteration in the charge distribution within the PPy nanoribbon, which occurred by reduction of the conductivity of the PPy nanoribbon, resulting in an improvement of the limit of detection down to 10 ng/ml. A further enhancement in the sensitivity was achieved by reducing the thickness of the PPy nanoribbon down to 25 nm. In addition, the dissolved salt concentration in the buffer solution that governed the solution Debye length screening effect, strongly affected the biosensing performance. The external charges of the biomolecules, which exceeded the solution Debye length, would be screened out. The investigation of these three factors along with integrated microfluidic channels, selection of monomer chemistries, as well as, their dopants would assist the design of conducting polymers based chemiresistive immunosensors with enhanced biosensing performance towards target analytes.
3.5 References


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Figure 3.1: A) Schematic illustration of LPNE process used for fabrication of PPy nanoribbon integrated with source and drain microelectrodes. B) Electrical measurement set-up for biosensing experiment.
Figure 3.2: Schematic diagram displaying fabrication of anti-CMV immobilized PPy nanoribbon based immunosensor to detect CMV (A). The schematic illustration is shown along with their corresponding I-V characteristics (B) of each step: 1.) the wet resistance of the PPy nanoribbon 2.) biofunctionalization of anti-CMV using carbodiimide (EDC) chemistry 3.) Prevention of non-specific binding by BSA blocking, and 4.) Addition of 0.1µg/ml CMV, respectively.
Figure 3.3: Calibration plots in terms of normalized resistance change of anti-CMV immobilized PPy nanoribbon based immunosensor for CMV detection with two different range of initial conductivity along with control experiment.
Figure 3.4: Calibration plots in terms of normalized resistance change of anti-CMV immobilized PPy nanoribbon based immunosensor in both 10mM phosphate buffer and phosphate buffer saline at pH = 7.4
Figure 3.5: Calibration plots in terms of normalized resistance change of anti-CMV immobilized PPy nanoribbon based immunosensor for CMV detection with thickness of 25 and 100nm
CHAPTER 4

SINGLE CHAIN FRAGMENT VARIABLE FUNCTIONALIZED SINGLE POLYPYRROLE NANORIBBON TOWARDS DETECTION OF PROTEIN MARKERS

Abstract

Herein, one-dimensional (1-D) polypyrrole (PPy) nanoribbon based bioaffinity chemiresistive nanosensor was used for identification and quantification of mycobacterium tuberculosis Ag85 complex. First, high affinity scFvs was selected using phage display techniques. The surface of PPy was biofunctionalized with single chain fragment variables (scFvs) specifically recognized Ag85 complex protein by N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) linking chemistry, followed by incubation of BSA to prevent non-specific binding. Detection of Ag85 complex protein was monitored by measuring the electrical resistance change. The sensors show excellent sensitivity, a low detection limit (i.e. 1-10 pM) depending on binding affinity of scFvs with wide dynamic range up to 10 nM and negligible signal towards unfunctionalized devices. In addition, our sensors show excellent specificity, which was insensitive towards other biomolecules including lysozyme. This work demonstrates the potential of biofunctionalized 1-D nanostructures as novel nano-biosensors for disease diagnostic.
4.1 Introduction

Detection methods based on immunological activity or merely interaction between antigen and antibody has been extensively used towards environmental monitoring, agriculture, and point-of-care (PoC) diagnosis extending from optical detection based on labeling antibodies with radioactive and fluorescent elements as well as enzymatic reaction in enzyme-linked immunosorbent assay (ELISA) format to immunoelectrochemical sensor (ELIECS). Fast and reliable immunodetection was further advanced by realization of label-free chemiresistive/FET immunosensors using one-dimensional (1-D) nanostructures owing to complementary innovative nanofabrication and assembly of high density 1-D nanostructure based nanodevices as well as their interesting chemical and physical properties that differed from bulk counterparts. First, they exhibited ultra-high surface area to volume ratio that promoted enhancement of molecular adsorption on their surfaces. The electrical transport through the bulk of 1-D nanostructures mitigated the current shunting nearby the accumulation/depletion of the charge carrier region caused by a molecular adsorption and interaction of charged biomolecules, improving limit of detection and boosting high sensitivity.

Recently, 1-D conducting polymers (CP) nanostructures such as polypyrrole (PPy), polyaniline (PANI), and poly(3,4 ethylenedioxythiophene) (PEDOT) appeared as promising functional materials for bio-electrical detection because they exhibited metal/semiconductor like properties, environmental stability, and biocompatibility, and retained flexibility and good mechanical property as polymers. They could be chemically/electrochemically polymerized under benign conditions where biomolecules can be simultaneously incorporated to generate specificity of biosensors. Their
monomers were derivatized by direct substitution functional groups that are suitable for well-established bioconjugation techniques.\textsuperscript{9,10} So far, the surface 1-D CP nanostructures were modified with antibodies to recognize specific target analytes such as CA-125, T7 and MS2 bacteriophages, showing an excellent detection limit.\textsuperscript{11,12}

Despite high specificity to target analytes, the typical size of antibodies is roughly 10 nm which exceeds the solution double layer thickness caused by a high salt concentration or high ionic strength in buffer solution.\textsuperscript{4} Thus, the interaction of antibodies-target outside this layer did not affect the charge distribution within the bulk of 1-D CP nanostructures due to the electrostatic screening effect.\textsuperscript{13} To overcome this challenge, small bioreceptors were exploited to ensure that interaction of bioreceptor-target occurred near the surface of the transducers. Recombinant single chain fragment variable antibodies (scFvs) have been alternatively employed as bioreceptors for label-free chemiresistive/FET immunosensors, which comprised of heavy ($V_H$) and light ($V_L$) chains of the variable regions of the antibodies linked by short flexible peptide chains.\textsuperscript{14} They also maintained a homogeneity and a high binding affinity for target recognition even if the constant region ($F_c$) was removed and the peptide linker was added.\textsuperscript{15} Isolation of recombinant scFvs could be achieved by display techniques such as phage display, ribosome and mRNA display, and microbial cell display\textsuperscript{16}, while the binding affinity of scFvs-target interaction could also be improved and optimized by several techniques such as mutagenesis and ribosome display.\textsuperscript{17} Furthermore, production of scFvs was normally done in bacteria cell cultures as compared to antibodies, which are normally produced in mammalian cell cultures.\textsuperscript{17} Hence, the reduction in size coupled with significant advantages provided facilitated the use of scFvs in the label-free chemiresistive immunosensor format.
In this work, we demonstrated PPy nanoribbon based bioaffinity chemiresistive immunosensor for detection of mycobacterium tuberculosis antigen 85 (Ag85) complex, which was the protein abundantly expressed this bacteria. The recombinant scFvs selected from phage display technique, screened, and depicted different binding affinity towards Ag85 complex would be employed as bioreceptors. Single PPy nanoribbon with defined 3µm gap microelectrodes was fabricated via lithographically patterned nanowire electrodeposition (LPNE) process. Ag85 specific scFvs and polyclonal antibody (pAbs) were incorporated onto surface of PPy nanoribbon via N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC)/N-hydrosuccinimide (NHS) chemistry. Sensing performance of Ag85 scFvs functionalized PPy nanoribbon was evaluated by I-V characteristics in absence and presence of Ag85 complex proteins ranging from 10 fM to 10 nM in phosphate buffer saline solution and spike human serum. Selectivity of sensors were validated by interaction of Ag85 specific scFvs with non-interactive biomolecule, lysozyme.

4.2 Experimental procedure
4.2.1 Device fabrication and assembly

Batch synthesis of single PPy nanoribbons (i.e., controlled dimension of 500 nm in width and 100 nm in thickness) with integrated gold microelectrodes were realized by using the LPNE technique as illustrated in our previous work. In brief, a sacrificial Ni layer with thickness of 100 nm was deposited onto a 4” thermally oxidized p-type Si wafer (i.e., 300 nm thick of SiO₂) (Ultrasil Corporation) using an e-beam evaporator. The wafers were passivated with adhesion promoter P20 (ShinEtsuMicroSi Microelectronic Material, Primer P20) followed by the photoresist (PR) 5214E (AZ Microelectronic
Materials) via spin coating at 1000 r.p.m. for 2s and 3000 r.p.m. for 30s. The wafers were then heated at 110°C on a hotplate for 5 min and exposed to an ultraviolet lamp with applied wavelength of 365nm and intensity of 5mW/cm² for 7s to photolithographically define a pattern. Subsequently, the wafers were soaked in a diluted developed solution (AZ Microelectronic Materials, 400K) and dried with ultra-high purity N₂ to complete the pattern transfer process. To produce the recessed Ni nanobands, selective Ni etchant (TFB Transene Company, Inc.) was used to chemically etch the exposed Ni film, and 0.1 M KCl + 24 mM HCl solution was employed to electrochemically etch Ni at an applied potential of 0.02 V vs. SCE. These conductive nanobands served as the conductive substrates for electropolymerization of PPy. The PPy nanoribbons were anodically electrodeposited at applied potential of 0.7 V vs. SCE in 0.5 M Py + 0.2 M LiClO₄ solution.

To integrate gold microelectrodes with 3µm gap onto the synthesized PPy nanoribbons, the wafers were submerged in an acetone bath for 5 min to dissolve PR. The same pattern transfer process was utilized to define aligned microelectrode patterns on the PPy nanoribbons. Ti as an adhesion layer and gold as an electrode material with thicknesses of 20 nm and 180 nm were sequentially e-beam deposited and the wafers were immersed in the acetone bath to get the integrated patterned microelectrodes. Afterward, 2% v/v HNO₃ solution was used to remove excess Ni, and the wafers were dried with high purity N₂ gas.

4.2.2 Biofunctionalization of PPy nanoribbon with scFvs and pAbs Ag85 antibodies

After device fabrication and assembly, the sample was mounted on a homemade Teflon incubation cell for well-improved fluid handling. The mixing solution of 20 µl of 40
mM N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDAC) (Sigma Chemical Co., St. Louis, MO. USA) in 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH = 5.5) and 200 µl of 10 mM N-hydrosuccimide (NHS) in dimethylsulfoxide (DMSO) (Thermo Fisher Scientific, Rockford, IL, USA) together with 20 µl of 45 µg/ml anti Ag85 scFvs or 7 µM pAbs anti Ag85B was added onto the incubation cell to establish an attachment of biorecognition molecules on the surface of the PPy nanoribbons via N-(3-dimethylaminopropyl)-N-ethylcarbodiimide(EDC)/N-hydrosuccinimide (NHS) chemistry. After 3 h incubation in a humidified container at room temperature, the solution was taken out. The surface of the PPy nanoribbons was washed with 10 mM PBS buffer solution containing 0.1% v/v Tween20 followed by PBS buffer solution for three times. The available active sites on the PPy nanoribbons’ surface were blocked by adding 150 µl of 1mg/ml BSA in PBS buffer for 1 h at room temperature, rinsed and washed by the same procedure as previously described.

4.2.3 Sensing Measurement

The sensitivity of the PPy nanoribbons was determined by probing the source (S) and drain (D) electrodes using the SOIC test clips wired to integrated Keithley switching and system sourcemeter 2636A (Keithley Instrument, Ohio) and sweeping the applied voltage from -0.5 to 0.5 V to generate the current-voltage (I-V) responses. The slope of these electrical responses in the linear region was inversed to calculate the electrical resistance of the PPy nanoribbons. Biotinylated Ag85 complex protein with the concentration of 100 nM was used as the experimental stock solution for further dilutions to various concentrations of 10fM to 10nM by PBS buffer solution. The electrical resistance of the PPy nanoribbons upon exposure to different concentrations of the
biotinylated Ag85 complex protein was determined in PBS buffer after incubation with 150 µl of the Ag85 complex protein solution, washing and rinsing with the protocol described earlier. The non-specific binding was investigated by incubation of various concentrations of 60 fM to 60 nM of lysozyme whereas the selectivity of the biosensor was evaluated by adding 20 µl of human serum (MP Biomedicals, Ohio) to the 100 nM experimental stock solution.

4.3 Results and discussion

Practically, label-free immunosensor detected and responses to changes in distribution of charge carriers within bulk of 1-D nanostructures from electric field induced by immunologic interactions. However, sensing performance of this sensor modality would be limited by screening effect from dissolved salt concentration in an aqueous solution which was defined as solution debye length ($\lambda_D$) given by

$$\lambda_D = \frac{1}{\sqrt{4\pi l_B \Sigma_i \rho_i z_i^2}}$$  \hspace{1cm} (1)

Where $l_B$ is the Bjerrum length = 0.7 nm, $\rho_i$ and $z_i$ are density and valence of ion species, respectively.\(^{19}\) Thus, to overcome challenges of this sensor configuration, small bioreceptors as scFvs would be alternatively employed.

Figure 4.1A-E illustrated the schematic diagram to fabricate anti Ag85 scFvs functionalized PPy nanoribbon based biosensor for Ag85 complex protein detection along with their corresponding I-V characteristics. PPy nanoribbons integrated microelectrodes (Figure 4.1A) were realized by LPNE technique which offered several advantages including a well control over device geometry via a combination of top-down photolithography and bottom-up electrodeposition approaches, no post alignment techniques required to position nanostructures on the substrates, and batch-scale
fabrication nature. The nanoribbons then functioned as a conduction channel for label-free chemiresistive biosensors. I-V characteristics of bare PPy nanoribbons were measured in the presence of PBS buffer solution, and the resistivity/conductivity of each PPy nanoribbon sample were obtained by inverting the slope in the linear region between -0.2 to 0.2 V and normalizing with PPy nanoribbon dimension. The resulting conductivity was 0.90±0.41 S/cm which is comparable to the conductivity range reported for 1-D PPy nanostructures.

Subsequently, PPy nanoribbons were functionalized with scFvs specific for Ag85 complex in Figure 4.1B using EDC chemistry where the carboxylic acid containing scFvs was activated by EDC/NHS to form carboxylate succimidyl ester, which underwent a nucleophilic substitution to generate a stable covalent attachment of scFvs on the surface of PPy nanoribbon. The zero linker molecule, EDC, also facilitated a direct linkage between PPy and scFvs without the presence of bifunctional linker molecules as depicted in surface functionalization schemes of inorganic materials or SWNTs. This ensured that the interaction of scFvs-Ag85 happened near the surface of PPy nanoribbon. As a result, the resistance increased owing to the charge localization under the bound region. The remaining active sites of the sensor devices were then blocked by BSA adsorption onto p-type PPy nanoribbon to prevent a non-specific binding which further raised the resistance because of the overall net negative charge exhibited by BSA (pI = 4.7) in PBS buffer (pH=7.4) in Figure 4.1C. Addition of 10pM of Ag85 complex onto the surface of anti-Ag85 scFvs functionalized PPy nanoribbon produced an increase in the resistance owing to inherent overall net negative charges of Ag85 complex in PBS solution(pH 7.4).
Prior to evaluation of the sensing performance of anti-Ag85 scFvs functionalized PPy nanoribbon, exposure of Ag85 complex protein to the unfunctionalized devices should be investigated as a control experiment. As shown in Figure 4.2, the normalized resistance changes ($\Delta R/R_0$) of the PPy nanoribbons were plotted as a function of Ag85 complex concentration in a log scale ranging from 10 fM – 10 nM where $R_0$ defined as the initial resistance measured in the buffer solution after BSA blocking, and $\Delta R$ signified the change of resistance measured at different exposed concentration of Ag85 complex proteins and initial resistance, $R_0$. The $\Delta R/R_0$ corresponding to unfunctionalized device (control) resulted in the change within 0.2 for the concentration ranging from 10 fM to 1 µM which was considered to be negligible $\Delta R/R_0$ responses to different exposed concentration of Ag85 complex.

After the control experiment was validated, two scFvs recognizing Ag85 complex proteins selected from a phage display technique, namely F1 and B1, whose binding affinities were obtained to be 94 nM and 14 nM, respectively, were functionalized on PPy nanoribbons to study the sensing performance with respect of binding affinity. Figure 4.2 depicted the sensing performance of recombinant scFvs F1 and B1 functionalized PPy nanoribbons towards detection of Ag85B complex proteins. The normalized change in resistance as a function of Ag85 complex concentration indicated that scFvs B1 exhibited similar responses as compared to scFvs F1 in all exposed Ag85 complex protein concentrations with the detection limit (LOD) of 100 pM. This result showed that the high binding affinity of engineered recombinant scFvs functionalized PPy nanoribbon biosensors strongly enhanced the sensing performance in terms of limit of detection.$^{26}$

Specificity of any biosensors is a critical factor to be assessed. Thus, the anti-Ag85 scFvs functionalized PPy nanoribbon was exposed to various concentrations of
lysozyme ranging from 60 fM-60 nM to study whether lysozyme had cross-interaction with scFvs F1. Prior to the sensor evaluation, the control experiment should be validated wherein lysozyme was introduced to unfunctionalized PPy nanoribbon based chemiresistive devices. As illustrated in Figure 4.3, $\Delta R/R_0$ was plotted as a function of lysozyme concentrations in log scale, and showed that $\Delta R/R_0$ increased and saturated within 0.6±0.09 to 0.13±0.05 from exposure of lysozyme at different concentrations between 60fM to 60nm. The response resembled the sensitivity response obtained from the control experiment performed with Ag85 complex proteins exposed to the unfunctionalized devices (Figure 4.2). This substantiated that the BSA passivated devices and considerably precluded non-specific binding of biomolecules. Subsequently, lysozyme was introduced to scFvs F1 functionalized PPy nanoribbons, and the sensing performance of scFv F1 functionalized PPy nanoribbon towards detection of Ag85 complex protein and lysozyme. Figure 4.3 showed the comparison of $\Delta R/R_0$ responses between detection of Ag85 complex protein and lysozyme. The negligible $\Delta R/R_0$ response towards detection of lysozyme using scFvs F1 functionalized PPy nanoribbon was observed when comparing with $\Delta R/R_0$ response obtained from detection of Ag85 complex protein using the same recombinant scFvs F1. This discrete response indicated the high specificity of recombinant scFvs F1 to Ag85 complex protein.

In addition, the sensing performance of scFvs functionalized PPy nanoribbons was compared with polyclonal antibodies (pAbs) specifically recognized Ag85B protein. In this study, the anti-Ag85 pAbs modified PPy nanoribbon based immunosensors were prepared using the same EDC/NHS surface functionalization scheme. Figure 4.4 illustrated the sensing performance of anti-Ag85B pAbs covalently immobilized PPy nanoribbons. The response of anti-Ag85B pAbs towards detection of Ag85 complex was
significant. As compared to the detection of Ag85 complex proteins using recombinant scFvs F1, the sensitivity response was lesser but line in the same range. The decrease in $\Delta R/R_0$ may be attributed to the effect of electrostatic screening which shielded the external charges outside the double layer of the buffer solution. Finally, scFvs functionalized PPy nanoribbons were exposed to the Ag85 complex proteins in the buffer solution containing the spike human serum. The response depicted selective detection of Ag85 complex protein using recombinant scFvs F1. This work showed the feasibility of Ag85 complex detection using recombinant Ag85 scFvs functionalized PPy nanoribbon based bioaffinity chemiresistive immunosensor, providing sensitive, selective, and simple diagnosis.

4.4 Conclusions

In summary, batch fabrication of single PPy nanoribbon integrated microelectrodes with 3 $\mu$m gap was realized by LPNE process. This sample was configured as bioaffinity chemiresistive immunosensor for detection of Ag85 complex. The specificity of the biosensors was accomplished by using engineered recombinant scFvs which specifically recognized Ag85 complex proteins. Sensing performance of this immunosensor was evaluated by normalize resistance changes as a function of various exposed Ag85 complex protein concentration in log scale both in PBS buffer and spike human serum. The control experiment was performed in unfunctionalized PPy nanoribbon, where the selectivity of the biosensors was evaluated by the interaction of scFvs Ag85 complex protein with lysozyme. The negligible responses resulted from non-specific binding to unfunctionalized and scFvs functionalized sensors. The sensitivity of Ag85 complex detection was depending on the binding affinity of scFvs. This study
demonstrated sensitive, selective and simple detection using 1-D conducting polymer based chemiresistive biosensor. This label-free bioaffinitiy based chemiresistive immunosensor could be further improved by implementing a microfluidic channel as well as engineering scFvs to exhibit functional binding domains for an oriented immobilization on transducers’ surfaces.
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Figure 4.1: Schematic diagram illustrating the construction of Ag85 scFv functionalized PPy nanoribbon based biosensor for Ag85 detection along with their corresponding I-V characteristics toward biological interaction on Ppy nanoribbon surface: A.) bare PPy nanoribbon B.) Immobilization of scFvs via EDC/NHS chemistry C.) Prevention of non-specific binding by ESA blocking, and D.) Addition of 10pM Ag85 complex.
Figure 4.2: Sensing performance toward Ag85 complex detection by two different Ag85 scFvs functionalized PPy nanoribbons.
Figure 4.3: Normalized change in resistance of scFv F1 functionalized single PPy nanoribbon based chemiresistive immunosensors for detection of Ag85 complex protein and lysozyme together with control experiment where lysozyme was exposed to unfunctionalized devices plotted as a function of Ag85 and lysozyme concentration in log scale.
Figure 4.4: Normalized changes in resistance of scFv F1 functionalized and anti-Ag85 pAbs functionalized PPy nanoribbons based chemiresistive immunosensor for Ag85 detection plotted with the normalized change in resistance of the unfunctionalized device served as the control experiment.
Figure 4.5: Comparison of normalized change in resistance of scFv F1 functionalized single PPy nanoribbon based chemiresistive immunosensors.
CHAPTER 5

ONE-DIMENSIONAL CONDUCTING POLYMER NANOWIRES BASED BIOAFFINITY CHEMIRESISTIVE SENSORS FOR DETECTION OF AG85B PROTEIN: EFFECT OF DOPANTS AND SOLVENTS

Abstract

PPy nanowires were synthesized by template-directed electropolymerization using various dopants ranging from small inorganic, ClO$_4^-$, and organic molecules, ToS, to large polymeric dopants, PSS, and solvents including water and acetonitrile. The difference in electrical conductivity was correlated by the dopants and solvents used. After maskless electrodeposition, ClO$_4^-$ depicted a drastic change in electrical property owing to a severe effect on electrochemical reduction and high ionic mobility. Longevity investigation also indicated that ToS doped PPy nanowires were the most stable among the synthesized PPy nanowires in this experiment. Therefore, ToS doped and ClO$_4^-$ doped PPy nanowires were selected for Ag85B protein detection and showed comparable sensitivity, selectivity, low detection limit of 1 pM, and dynamic range spanning up to 10 nM, where the diameter of nanowires can tune the sensing performance towards detection of Ag85B protein.
5.1 Introduction

Conducting polymers (CP) have been attractive functional molecular building blocks for advanced potential applications especially chemiresistive/FET chemical/biological sensors because they enabled direct conversion between chemical/biological interactions and electrical signals. Their monomers could be derivatized with suitable functional groups for direct covalent functionalization. The versatility in bioconjugation methods such as chemical and electrochemical entrapment during polymerization under benign synthesis conditions as well as their biocompatibility stimulated utilization of CP based biosensors. In addition, confining these nanostructures to one-dimensional (1-D) nanoscale further enhanced the detection limit as well as the sensitivity owing to the electronic conduction which appears in the bulk of 1-D nanomaterials and comparable size to their debye screening length. Therefore, these features allowed a significant effect in charge depletion/accumulation region, resulting in a large change in electrical conductance/resistance upon chemical/biological interactions on the surface of 1-D conducting polymer nanomaterials.

Recently, 1-D CP nanostructures have been configured as chemiresistive/FET chemical/biological sensors for detection of various biomolecules including ions, proteins, viruses, and oligonucleotides with excellent sensitivity and low detection limits. These nanostructures have been synthesized by using only common spherical inorganic dopants such as Cl\(^-\) and ClO\(_4^-\). Nevertheless, previous gas sensing studies have illustrated that solvent, and dopants strongly affect the structure of conducting polymers (i.e., conjugation length and polydispersity) and their electrical properties which ultimately influenced the sensing performance. Therefore, a proper selection of dopants and solvents also became critical to maintain conductivity and
stability of dopant/conducting polymers interactions for chemiresistive/FET biosensing applications.

Various fabrication techniques have been developed to synthesize conducting polymer nanowires and position them to a well-defined location such as e-beam lithography (EBL)\textsuperscript{12} and lithographically patterned nanowire electrodeposition (LPNE) technique.\textsuperscript{13,14} Even though these techniques provided precise controls over a device geometry and bypassed post device assembly, they still had limitations. EBL was expensive equipment that offered a serial process to the device fabrication. In addition, even though LPNE technique eliminated the use of EBL patterning and supported the cost-effective bulk scale synthesis of 1-D CP based electronic devices, the organic solvents such as acetonitrile, which promoted the conjugation length by preventing nucleophilic attack during polymerization, were not able to be used for polymerization. Therefore, template-directed electrodeposition technique which confined the oxidation of monomers and chemical/electrochemical polymerization in hard porous templates such as alumina and track-etch polycarbonate templates with a well-defined pore size,\textsuperscript{15} alternatively became a facile and economical approach to synthesizing 1-D CP nanostructures for the solvent and dopant depended investigation.

Herein, PPy nanowires with diameter of 30 nm were synthesized via template-directed synthesis method and configured as a chemiresistive device for detection of Ag85B proteins that expressed by mycobacterium tuberculosis.\textsuperscript{16} Various dopants and solvents were employed during PPy electropolymerization to investigate their effects towards the electrical property and stability in an ambient air as well as in the PBS buffer solution. \(N\)-(3-dimethylaminopropyl)-\(N'\)-ethylcarbodiimide (EDC)/\(N\)-hydrosuccinimide (NHS) chemistry was also used to covalently anchor polyclonal antibodies, anti-Ag85B,
that specifically recognized Ag85B proteins on the PPy nanowires. The sensing performance of anti-Ag85B modified PPy nanowires in terms of sensitivity and selectivity will be evaluated.

5.2 Experimental procedure

5.2.1 Synthesis of PPy nanowires

PPy nanowires were electrochemically synthesized via well-established template-directed electrodeposition method using laboratory fabricated 30 nm pore diameter alumina membranes as templates. These templates were electrochemically synthesized using an electrochemical cell with a two-electrode configuration setup in 1.8 M H$_2$SO$_4$. The working electrode was 1.27 mm aluminum sheet, while Pt-coated Ti strip was used as the counter electrode. Prior to membrane fabrication, the surface of aluminum was treated with 1 M NaOH and subjected to anodizing at an applied potential of 15 V for 30 min followed by 20 V for 90 min using Agilent E3647A Dual Output DC power supply (Agilent Technologies, Inc. Santa Clara, CA). The solution was rigorously stirred and kept at 20°C throughout the anodization. After the process got terminated, an excess aluminum was etched by 0.1 M CuCl$_2$ + 2.4 M HCl solution, and the alumina was then submerged in 0.74 M H$_3$PO$_4$ solution for 30 min during the pore widening step. The alumina was rinsed 3 times with nanopure water and air dried. All the chemicals listed above were all reagent grades and purchased from Fisher Scientific. The conductive seed layer was deposited by sputtering 200 nm gold layer on one side of the alumina template using EMS 575X sputter (Electron Microscopy Science, Hatfield PA).

The different dopants including LiClO$_4$ (Fisher Scientific), p-toluenesulfonic acid (p-ToS) (Sigma-Aldrich, St. Louis, MO), p-toluenesulfonic acid, sodium salt (Sigma-
Aldrich, St. Louis, MO) and sodium polystyrene sulfonate (NaPSS, MW 70000) (Sigma-Aldrich, St. Louis, MO) along with nanopure water and acetonitrile (Fisher scientific) as solvents used as indicated in the table 5.1. The dopant solution with a concentration of 0.1 M was deoxygenated with ultra-high purity N\textsubscript{2} for 30 min, and 0.1 M distilled pyrrole (Py) monomer solution (Sigma Aldrich, St Louis, MO) was subsequently added and thoroughly mixed for 15 min prior to use. PPy nanowires were electropolymerized by chronoampermetry in a 100ml cell with three-electrode configuration setup. The constant potential of 1.0 V vs. SCE (or Ag/Ag\textsuperscript{+}) was applied with Pt-coated Ti strip as the counter electrode. The electrochemical reaction was terminated when a passing charge density reached 5.18 C/cm\textsuperscript{2}. The selective gold etchant (0.15 M KI in a 0.1 N I\textsubscript{2}) was used to remove the gold seed layer, followed by washing the alumina template with nanopure water and dissolving it overnight in 4.4 M H\textsubscript{3}PO\textsubscript{4}. The nanowire suspension was diluted 5-fold with the same H\textsubscript{3}PO\textsubscript{4} solution, centrifuged at 13,200 rpm for 30 min, and washed with nanopure water. The washing step was repeated twice, and the PPy nanowires were then suspended in nanopure water or 50/50 methanol-water mixture in a 1.5 ml microcentrifuge tube, sonicated and centrifuged again to collect 600mL supernatant for the fabrication of PPy nanowires based device.

5.2.2 Fabrication and assembly of PPy nanowires based device

Pre-fabricated microelectrodes were prepared by a standard lift-off photolithography technique. First, an adhesion primer (ShinEtsuMicroSo Microelectronic Material, Primer P20) and a subsequent positive photoresist 5214E (AZ Microelectronic Materials) were spin coated on a thermally oxidized commercial Si wafer (Ultrasil Corporation) with an oxide thickness of 300 nm at a rotation speed of 1000 rpm for 2
sec, followed by that of 3000 rpm for 30 sec and baked on a hotplate for 5 min at 110°C. The microelectrode pattern was defined by exposing the wafers to UV lamp at a wavelength of 365 nm and intensity of 5mW/cm² for 9 sec, and developed by submerging the wafer in the mixture of water and developing solution AZ 400K (AZ Microelectronic Materials) in 4:1 ratio for 30 sec. Ti as an adhesion and Au with thickness of 20 nm and 180 nm were sequentially deposited at rates of 1Å/s and 3Å/s, respectively using a Tamescal BJD-1800 e-beam evaporator. The microelectrodes were then lifted off by soaking in acetone bath overnight, and dried with high purity N₂. Each pre-fabricated microelectrode chip consisted of 15 pairs of the gold electrodes with 3 μm gap.

Alternating current (AC) dielectrophoretic alignment technique was employed to position PPy nanowires onto the prefabricated microelectrodes. The well-dispersed suspension of PPy nanowires (400μl in volume) was dropped onto the electrode mounted to the customized electrochemical cell, and AC field of 50 kHz frequency and 5 V peak-to-peak voltage between two terminals was applied for 1 min using Keithley 3390 50 MHz Arbitrary Waveform Generator (Keithley Instruments, Inc. Cleveland, OH), and the electrode was washed with water. Maskless electrodeposition technique was subsequently deployed to anchor the PPy nanowires to the gold electrodes. The three-electrode configuration electrochemical cell was used, which comprised of all electrode pairs, were shorted by a copper tape and conductive silver (Ted Pella, Inc. CA) to form a single working electrode, a Pt wire as the counter electrode, and Ag/AgCl as the reference electrode. The electrolyte for the gold maskless electrodeposition was Technigold 25 ES RTU (Technic Inc., CA) at pH of 7.0. The constant potential of -0.9 V vs. Ag/AgCl was applied with a controlled charge density of 0.06 C/cm² to anchor the
PPy nanowires. The samples was rinsed with nanopure water and dried with high purity \( \text{N}_2 \) gas.

5.2.3 Stability in an ambient air and the presence of PBS buffer solution (pH=7.4) and non-specific binding studies

For investigation of longevity in the air and the presence of PBS buffer solution, the electrical resistances of the various doped PPy nanowires were achieved by inversing the slopes of the I-V responses obtained from sweeping the applied voltage of -0.5 to 0.5 V using the integrated Keithley switching and system sourcemeter 2636A (Keithley Instrument, OH). The electrical resistances of the samples were measured daily in the first week and once per week in the following weeks.

For the study of non-specific binding, 150\( \mu \)l of 1mg/ml Bovine Serum Albumin (BSA) (Sigma-Aldrich Inc., MO, USA) in PBS solution was put on the various doped PPy nanowire based devices which were mounted onto the homemade Teflon incubation cell, and incubated for 1h. The BSA solution was drawn out, and the samples were washed with 10mM PBS solution (pH = 7.4) containing 0.1% v/v Tween20 and rinsed with PBS. The procedure was repeated three times. The electrical resistances before and after BSA adsorption were measured by the same measurement described previously.

5.2.4 Biofunctionalization of PPy nanowires with polyclonal Ag85B antibody

\( N-(3\text{-dimethylaminopropyl})-N\text{-ethylcarbodiimide(EDC)/N-hydrosuccinimide} \) (NHS) chemistry was employed to produce an attachment of polyclonal Ag85B
antibodies (Abcam, Cambridge, MA, USA) on the surface of the PPy nanowires. The biofunctionalization process was achieved by incubating the PPy nanowires with 20 µl of various concentration of anti-Ag85B (i.e., 1 µM, 7 µM, 14 µM), 20 µl of 40 mM N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) (Sigma Chemical Co., St. Louis, MO, USA) in 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH = 5.5) and 200 µl of 10 mM N-hydrosuccimide (NHS) in dimethylsulfoxide (DMSO) (Thermo Fisher Scientific, Rockford, IL, USA) to generate a stable intermediate carboxylate succimidyl ester and later chemically react with the active amine group on the surface of the PPy nanowires for 3 h. Subsequently, the solution mixture was aspirated out, and the devices were washed as stated previously. The PPy nanowires based sensors were incubated with 1 mg/ml BSA in PBS buffer solution to block the non-specific binding. After 1 h incubation, the BSA solution was pipetted out, washed, and rinsed by following the same protocols described previously.

5.2.5 Sensing measurement

The 100 nM aliquots of Ag85B were prepared as the experimental stock solutions for further dilutions to various Ag85B concentrations ranging from 10 fM to 10 nM for the sensing studies. The 10 µM aliquots of lysozyme were prepared as the experimental stock solutions for further dilutions to various lysozyme concentrations of 1 pM, 1 nM, and 1 µM for the control experiment.

The electrical resistance of the PPy nanowires upon exposure to different concentrations of Ag85B was measured in the PBS buffer solution after the incubation with 150 µl of Ag85B solutions, and the washing and rinsing protocols. The same
sensing procedure was applied to the non-specific binding experiment towards an exposure of lysozyme.

5.3 Result and discussion
5.3.1 Synthesis of nanowires and device assembly

Five different PPy nanowires synthesized by template-directed electrodeposition were used to investigate material properties depended sensing performance towards detection of Ag85B protein. The abbreviation of PPy nanowires synthesized from different dopants and solvents were listed in table 5.1 and would be used henceforth. By controlling a charge density, the resulting nanowires exhibited the length approximately 15.08±1.26 µm and retained their length after the rigorous sonication. The conductivity of each PPy nanowire sample was obtained after AC dielectrophoretic alignment onto 3 µm gap pre-fabricated microelectrodes and listed in table 5.1. Even though the resulting conductivities lied within the reported electrical conductivity of PPy nanowires\textsuperscript{18,19}, the discrepancies among different PPy nanowires could be attributed to dopant types ranged from small inorganic to large polymeric anions as well as solvent (i.e., water and acetonitrile) used which mainly affected the conjugation length and packing arrangement.\textsuperscript{20}

First, a solvent effect towards polymer structures was considered by comparing between PTSA and PTSAW or PCA and PCAW. The nanowires synthesized using water as solvent generally exhibited lower electrical conductivity due to the nucleophilicity of water. PPy nanowires synthesized underwent a nucleophilic attack which terminated chain growth and caused an irregular morphology or surface defects in the polymers.\textsuperscript{21} The low conjugation length and surface defects prohibited a charge delocalization within
the polymer, resulting in the low electrical conductivity as observed in PTSAW and PCW.\textsuperscript{22} On the other hand, acetonitrile was a highly aprotic solvent which could minimize the nucleophilic attack during the polymer growth. This organic solvent generally produced the polymers with a well packing arrangement, and a long conjugation length to facilitate the electron transport or charge delocalization as imparted in PTSA and PCA.\textsuperscript{21}

Secondly, dopant size and nature significantly influenced the characteristics of conducting polymers. To investigate the dopant effects, small dopants such as p-toluenesulfonate (ToS), and perchlorate (ClO\textsubscript{4}\textsuperscript{-}), and a large polymeric dopant such as polystyrene sulfonate (PSS) (MW ~ 70000) were used in this study. For the consistency, the conductivity of PTSAW, PCW, and PSSW were compared, because they were all synthesized from the water bath. As seen in table 5.1, the conductivity of PSSW exhibited the lowest conductivity while that of PTSAW resulted in the highest conductivity among these three PPy nanowires. The low conductivity of PSSW was derived from a large polystyrene sulfonate (PSS) in which partial charges on the PSS backbone effectively doped PPy fragments.\textsuperscript{23} Remaining charges trapped in the PPy did not contribute to the conductivity of PSSW. The high conductivity observed in PTSAW and PCW were resulting from a small size of dopant anions that intercalated into the polymer layers, where the slightly higher conductivity in PTSAW originated from a planar aromatic structure that promoted a high level of preferred orientation of the pyrrole rings parallel to the electrodes or growth surface.\textsuperscript{24}

Afterward, the improvement of electrical contacts was realized by masklessly electrodepositing gold at a constant applied potential of -0.9 V vs. Ag/AgCl and controlled charge density of 0.06 C/cm\textsuperscript{2} on the gold microelectrodes to embed the ends
of PPy nanowires, protecting nanowires from a fluid handling during the biosensing experiments. This condition selectively deposited gold on gold microelectrodes. The normalized change in electrical resistance ($\Delta R/R_0$) of various doped PPy nanowires were shown in Figure 5.1, where $R_0$ and $R$ were defined as the initial resistance and resistance measured after the maskless electrodeposition in ambient air, respectively. The drastic increase in $\Delta R/R_0$ could be clearly observed in PPy nanowires synthesized from an acetonitrile bath. This implied that a severe reduction occurred during the maskless electrodeposition.\textsuperscript{17} Figure 5.1 also depicted that ClO$_4^-$ doped PPy had significant impact to the maskless electrodeposition, owing to a high ionic mobility, where PSS became the most stable under the electrochemical reduction because of an immobility of large polymeric anion.\textsuperscript{11}

### 5.3.2 BSA adsorption

After maskless electrodeposition, five different PPy nanowire based chemiresistive devices were exposed to BSA, and the electrical resistances were measured before and after BSA incubation. Figure 5.1 also depicted the percentage of changes in the electrical resistance of PPy nanowires. A considerable change in the electrical resistance has clearly been observed in ClO$_4^-$ doped PPy nanowires whereas less change in the electrical resistance was found in ToS and PSS doped PPy nanowires, suggesting that different dopants influenced BSA adsorption. Minimal change in the electrical resistance of PSS towards BSA adsorption was attributed to excessive charges on the polyanions that did not contribute to doping process, making the surface become more hydrophilic than the other investigated PPy nanowires.\textsuperscript{23}
In addition, protein adsorption could also be affected by an electrostatic interaction. BSA (pI =4.7) normally exhibited overall net negative charges in PBS buffer solution. Therefore, owing to high ionic mobility of ClO$_4^-$, negatively charged BSA could interact with oxidized polymer backbone that led to an extensive increase in electrical resistance as compared to that of PSSW.

5.3.3 Conductivity of doped PPy in an ambient air and PBS buffer solution

To investigate the longevity in an ambient air, doped PPy nanowires were stored in a dessicator covered with Al foil overnight to prevent light exposure and remove moisture contents from the preceding maskless electrodeposition and sample cleaning. After an overnight storage, their electrical resistances were measured, which was denoted as the initial resistance ($R_0$). On the other hand, doped PPy nanowires samples were directly mounted onto a homemade Teflon cell after the prior sample preparation. The initial resistance ($R_0$) was obtained once 150 µl PBS buffer solution was added. The resistances were monitored daily in the first week and once in the following weeks. All the samples were stored in a dessicator (for an ambient air) and a humidified chamber (for PBS solution) between the electrical resistance measurements. The normalized change in the resistance ($\Delta R/R_0$) both in ambient air and in PBS buffer solution were determined and plotted in Figure 5.2.

An increase in $\Delta R/R_0$ to approximately 0.36±0.18 of PSSW was observed in ambient air by the end of the first week where the other doped PPy nanowires imparted 0.1-0.2 in $\Delta R/R_0$ (Figure 5.2A). The $\Delta R/R_0$ of PSSW was stabilized and gradually increased to nearly 0.5 in the following week. The decrease in $\Delta R/R_0$ of PTSA, PTSAW, PCA, and PCW were seen in the second week after storage in the dessicator for a
couple of days and continued to be stabilized within 0.1 in the following week. On the contrary, every sample stored in PBS buffer solution showed the increase in $\Delta R/R_0$. The drastic increase in $\Delta R/R_0$ to 4 of PSSW was also illustrated. The severe degradation of PSSW continued in the second week, and the electrical resistance became extremely too resistive to be measured in the buffer solution thereafter. This degradation could be sequenced from the most unstable down to the most stable dopant employed in this study as PSSW>PCW>PTSAW>PCA>PTSA, where PTSA depicted the greatest stability among all of the PPy samples.

The degradation of electrical properties of PPy nanowires in the ambient air was influenced by hydrophilic/hydrophobic nature of dopants. Hydrophilic dopants such as polyanionic dopant or PSS in this investigation promoted an adsorption of moisture in the ambient air, leading to a polymer swelling which increased packing disorder and restricted charge carrier movements. Moreover, a nucleophilic attack by oxygen at β-position of polycationic chain eventually yielded a formation of carbonyl structures which induced a shortening of the conjugation length and therefore increased the electrical resistance of PPy nanowires. Similarly, a strong degradation and concomitant increase in $\Delta R/R_0$ of all PPy nanowire samples especially PSSW was clearly observed in PBS buffer solution as illustrated in Figure 5.2B. Besides the swelling and nucleophillic attack by water molecules (OH⁻) described earlier, Fonner et al. indicated that the doping interaction between PSS and PPy was irreversibly lost over time even if PSS was trapped within the PPy nanowires in the water. In the presence of PBS buffer solution, Fonner et al. also suggested that ions could potentially displace the dopant ions or quenched the interactions between PPy and dopants. All these factors thus contributed to the degradation of PPy nanowires.
5.3.4 Sensing performance of Ag85B

Based on stability investigation, PTSA and PCW were selected to fabricate bioaffintly chemiresistive based sensors for detection of Ag85B protein. Antibody (Ab), anti-Ag85B, was anchored on the surface of PPy nanowires using EDC/NHS chemistry with Ab loading concentration of 7 µM. The devices were incubated with BSA to block the available active sites on the nanowires. The sensitivity of these nanowire immunosensors would be evaluated in terms of the normalized change in electrical resistance ($\Delta R/R_0$) as a function of Ag85B protein concentration in 10 mM PBS buffer where $R_0$ represented the initial electrical resistance after BSA blocking step, and $R$ was the electrical resistance after exposure to Ag85B protein. Non-specific binding (NSB) of nanowire immunosensors was evaluated by exposure of lysozyme onto anti-Ag85 immobilized PCW and PTSA at concentration of 1 pM, 1 nM, and 1 µM. As shown in Figure 5.3C and 5.3D, $\Delta R/R_0$ of PCW and PTSA were 0.04±0.09 and 0.04±0.06 after exposure of 1 pM of lysozyme, and remained at 0.08±0.06 and 0.11±0.07 after subsequent exposures, respectively. With NSB binding taken into account, anti-Ag85B immobilized PCW and PTSA imparted the limit of detection (LOD) of 1 pM and wide dynamic range up to 10 nM with comparable $\Delta R/R_0$, demonstrating the feasibility of PTSA as a more stable transducer.

Various Ab concentrations of 1 µM, 7 µM, and 14 µM were investigated to study this effect towards the sensing performance using PTSA as illustrated in Figure 5.4. By reducing the Ab loading to 1µM, the sensor produced negligible $\Delta R/R_0$ responses which suggested an insufficient amount of immobilized Ab on the surface. The increase in Ab concentration to 14 µM depicted higher $\Delta R/R_0$, since more Ag85B protein could be
captured by anti-Ag85B immobilized onto surface. In addition, as previously suggested, reduced dimension of transducer, optimized device geometry, and controlled doping concentration can significantly enhance the sensing performance induced by the electrostatic screening effect. Therefore, PTSA with diameter of 200 nm was used as a comparison to the one with 30 nm. The Ab loading was kept constant at 7 µM. Figure 5.5 showed the effect of diameter of PTSA dependent sensing performance towards detection of Ag85B protein. Decrease in \( \Delta R/R_0 \) resulted from increasing PTSA nanowire diameter at the same Ag85B protein concentration range. Nevertheless, the PTSA with diameter of 200 nm was anticipated to provide longer dynamic range, implying that more surface charges were required to fully deplete/accumulate the conduction channel. These sensing results denoted the feasibility and applicability of the conducting polymer based bioaffinity chemiresistive sensors for detection of Ag85B protein by tailoring the device dimension and anionic dopants.

### 5.4 Conclusion

In summary, PPy nanowires with various dopants as \( \text{ClO}_4^- \), ToS, and PSS were synthesized via a simple and robust template-directed electropolymerization in different solvents including water and acetonitrile. Nanowire devices were realized by using AC dielectrophoretic alignment to position nanowires bridging the microelectrode gap, followed by the maskless electrodeposition to ensure good electrical and mechanical contacts. Increase in resistance after embedding both ends of PPy nanowires, especially in \( \text{ClO}_4^- \), was due to high ionic mobility, where PSS depicted high resistance to electrochemical reduction. Afterwards, all PPy samples were subject to a longevity experiment where PSSW degraded in ambient air and severely in PBS buffer as
compared to others, possibly due to the hydrophilic nature of dopant and low conjugation length. By this investigation, PTSA and PCA were chosen for Ag85B protein detection. The comparable sensing performance in terms of sensitivity, selectivity, dynamic range, and limit of detection of 1pM of Ag85B protein were obtained where PTSA appeared highly stable in PBS buffer as compared to PCA. In addition, the sensitivity can be enhanced via controlling device geometry such as the nanowire diameter.
5.5 References


(7) Bangar, M. A.; Shirale, D. J.; Chen, W.; Myung, N. V.; Mulchandani, A. *Analytical Chemistry* 2009, 81, 2168-2175.


Table 5.1: Summary of dopants and solvents use for synthesis of PPy nanowires and resulting conductivity after AC dielectrophoretic alignment

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Concentration of pyrrole (Py) monomer and dopants</th>
<th>Solvent</th>
<th>Conductivity (S/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA</td>
<td>0.1M Py + 0.1M LiClO$_4$</td>
<td>Water</td>
<td>1.50±0.76</td>
</tr>
<tr>
<td>PCW</td>
<td>0.1M Py + 0.1M LiClO$_4$</td>
<td>Acetonitrile</td>
<td>0.04±0.02</td>
</tr>
<tr>
<td>pTSA</td>
<td>0.1M Py + 0.1M pTSA</td>
<td>Acetonitrile</td>
<td>1.23±0.35</td>
</tr>
<tr>
<td>pTSAW</td>
<td>0.1M Py + 0.1M pTSA</td>
<td>Water</td>
<td>0.14±0.10</td>
</tr>
<tr>
<td>PSS</td>
<td>0.1M Py + 0.1M NaPSS</td>
<td>Water</td>
<td>0.007±0.002</td>
</tr>
</tbody>
</table>
Figure 5.1: Change in the PPy nanowires’ resistances after maskless electrodeposition (ME) and BSA adsorption (BSA).
Figure 5.2: Normalized change in the resistance ($\Delta R/R_0$) of various doped PPy nanowires in ambient air (A) and in the PBS buffer solution (B) measured within two weeks.
Figure 5.3: Normalized change in resistances of anti-Ag85B functionalized PCW and PTSA nanowires for Ag85B protein detection (A and B) and non-specific lysozyme binding (C and D) as a function of Ag85B and lysozyme concentrations plotted in log scale, respectively.
Figure 5.4: Normalized change in the resistance ($\Delta R/R_0$) to exposure of various concentration of Ag85B protein using PPy nanowires with different diameter (i.e., 30 and 200nm).
Figure 5.5: Normalized change in the resistance ($\Delta R/R_0$) to exposure of various concentration of Ag85B protein using PPy nanowires with different diameter (i.e., 30 and 200nm).
CHAPTER 6

CONCLUSION AND FUTURE DIRECTIONS

Recently, the development of field deployable, label-free, ultra-sensitive sensory devices that enable reliable and direct electrical detection has been intensively researched to overcome drawbacks associated with traditional optical and electrochemical methods. Advancement of nanotechnology and biotechnology has prompted utilization of 1-D nanostructures as a novel sensing platforms, owing to their ultra-high surface area to volume ratio, size dependent electrical properties, and possibility of a device miniaturization.

Conducting polymers (CP) are π-conjugated system which has single and double bonds alternating along the polymer chain. They emerged as another class of materials for chemiresistive/chemFET biosensors because they exhibit remarkable optical, magnetical, and electrical properties like a semiconductor while possessing mechanical properties and ease of fabrication as polymers. Tunable electrical conductivity can be realized by several orders of magnitude (i.e., $10^{-10}$ to $10^3$ S/cm) via the process called ‘doping’ where anion is chemically or electrochemically incorporated to oxidized CP backbone to attain the charge neutrality. Amongst CPs, polyheterocycles such as polypyrrole (PPY), polyaniline (PANI), poly(3,4-e-thylenedioxythiophene) (PEDOT) and their derivatives are used as transducers due to great environmental stability, high conductivity, and functional monomers for direct immobilization of the bioreceptors by various surface immobilization routes such as physical adsorption, entrapment, cross-linking, and covalent bonding.
Thus, CP nanowires/tubes configured as chemiresistors and FETs have been developed as analytical tools for detection and monitoring of various chemical and biological molecules. While this nano-electronic biosensor illustrates strong potential for advanced technology in detection and monitoring, limitation in device fabrication and assembly for high density sensor arrays appears challenging to be scalable and reproducible in a cost effective manner.

Hence, the overall objective of this research work concentrates in the fabrication and development of 1-D conducting polymer nanostructures for chemiresistive/FET chemical and biological sensors. LPNE and template-directed electrodeposition techniques have been employed to synthesized 1-D CP nanostructures with controlled dimensional, physical, chemical, and electrical properties. Various capture probes such as polyclonal antibody (pAbs) and single chain fragment variables (scFvs) were investigated, and sensing performance in terms of sensitivity, dynamic range, and limit of detections towards specific targets was evaluated.

6.1 Summary

In this context, Electrical biosensors, the sensing methodologies, and the predominating sensing mechanism (i.e., electrostatic gating effect and schottky barrier height modulation) especially for chemiresistive/FET sensors were discussed. The 1-D nanomaterials that could potentially be employed for these sensors including were summarized in chapter1 including their fabrication methods, surface functionalization, and recent application of 1-D based sensors.
Based on the review and discussion in chapter 1, batch-scale synthesis of ultra-long 1-D conducting polymer nanostructures or PPy nanoribbons with controlled dimension and position on the substrates were realized by using LPNE technique.

Ultra long (> 1 cm) polypyrrole (PPy) nanoribbons were batch-synthesized to predetermined locations on 4 inch Si wafers and flexible polyimide film with controlled thicknesses and widths via modified Lithographically Patterned Nanowire Electrodeposition (LPNE), which combines top-down “photolithography” and bottom-up “electrodeposition”. The excessive lengths and lithographic approach enable subsequent patterning of electrodes for direct device integration. Electrical transport properties were investigated by temperature dependent measurements, and back-gated FET measurements were performed to study the properties with respect to the conductivity of PPy nanoribbons. To demonstrate the utility of this approach these nanoribbon devices were interrogated as gas sensors in a conductometric fashion. They displayed excellent sensitivity toward ammonia vapor with a detection limit in the sub ppm. The sensitivity toward ammonia vapor was tuned by the conductivity of the PPy nanoribbons via chemical de/doping.

LPNE was used to fabricate one dimensional conducting polymer nanostructures with integrated microelectrodes for sensing applications. The fabrication technique combines attributes of photolithography and electrodeposition into one step, synthesizing high aspect ratio conducting polymer nanoribbons to precise locations on silicon/silicon dioxide substrates with controlled dimensions. The process permits these cm long nanoribbons to be readily interfaced with existing microfabrication technologies, which distinguish individual devices by their placement, offering a manufacturable method to fabricate nanostructured conducting polymers. Temperature dependent
electrical properties of the PPy nanoribbons exhibited semiconductor properties based on the negative TCR and activation energies. These nanoribbons were also shown to make excellent gas sensors with detection down to sub-ppm concentrations of NH₃ at room temperature. The sensitivity toward ammonia gas was tuned by chemically modulating the conductivity of PPy nanoribbons.

6.2 Future Directions
Sensing performance of CP based biosensor can be realized by tailoring the structure and properties of PPy to maintain stability and still provide functionality towards specific detection of biomolecules.
1. Improvement of CP based biosensors, especially PPy can be done by derivatize Py monomer to block the undesired side reaction in the presence of moisture.
2. Hybrid systems can be incorporated to together with engineered bioreceptors that can provide multiplexing