INTEGRATED CHEMICAL AND BIOLOGICAL SYSTEMS

IN NANOWIRE STRUCTURES TOWARDS NANO-SCALE SENSORS

A Thesis in
Materials
by
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ABSTRACT

Nanowires composed of metal and conducting polymers with integrated proteins and chemical systems have been investigated as building blocks for next-generation nano-scale sensors and assemblies. These nanowires were fabricated by combining chemical and electrochemical methods of synthesis of gold and conducting polymers in nanopores of anodized alumina membranes. Polymer nanowires were synthesized from buffer solutions as a mean to promote a biocompatible environment for the incorporation of proteins. A variety of proteins were incorporated into the polymer matrix by entrapment during polymerization that imparted the polymer material with biological functionality. Another class of composite nanowires containing electro-active conducting polymer junctions was developed for applications in chemical sensor arrays. The methodologies described in this thesis provide an inexpensive and straightforward approach to the synthesis of anisotropic nanoparticles incorporating a variety of biological and inorganic species that can be integrated to current microelectronic technologies for the development of nano-scale sensor arrays.
# TABLE OF CONTENTS

List of Figures .................................................................................................................. viii
List of Tables ................................................................................................................... xvi
Acknowledgments ........................................................................................................... xvii

Chapter 1: Introduction ...................................................................................................... 1
  1.1 Brief Overview of Nanoparticle Technology .......................................................... 1
  1.2 Overview of Fabrication Techniques for Nanowire Assemblies ......................... 2
  1.3 Nanowire Assemblies ........................................................................................... 4
  1.4 Nanowire Sensor Model ....................................................................................... 5
    1.4.1 Brief Overview of Conducting Polymers ..................................................... 7
  1.5 Objectives and Organization of the Thesis ........................................................... 7
  1.6 References .......................................................................................................... 10

Chapter 2: Fabrication and Characterization of Conducting Polypyrrole Nanowires .... 18
  2.1 Introduction .......................................................................................................... 18
    2.1.1 Properties of Polypyrrole .......................................................................... 18
    2.1.2 Polypyrrole Nanowires and Nanotubes ................................................... 19
  2.2 Experimental Methods ......................................................................................... 21
    2.2.1 Materials ................................................................................................... 21
    2.2.2 Characterization ......................................................................................... 21
    2.2.3 Electrochemical System ............................................................................. 22
    2.2.4 Synthesis of Conducting Polypyrrole Films .............................................. 22
    2.2.5 Synthesis of Polypyrrole Nanowires ....................................................... 23
    2.2.6 Synthesis of Segmented Metal/polymer/metal Nanowires ......................... 25
  2.3 Results and Discussion ......................................................................................... 25
    2.3.1 Polypyrrole Films and Nanowires: Monomer Concentration and Solvents 25
    2.3.2 Polypyrrole Nanowires: Electrochemical Method of Synthesis ................ 28
    2.3.3 Polypyrrole Nanowires: pH Dependence .................................................. 31
    2.3.4 Polypyrrole Nanowires: Oxidation Potential ............................................. 32
2.3.5 Segmented Au/Polypyrrole/Au Nanowires ................................................. 33
2.4 Conclusions ............................................................................................................ 35
2.5 References .............................................................................................................. 36

Chapter 3: Protein-modified Polypyrrole Nanowires ............................................. 59
3.1 Introduction ............................................................................................................ 59
3.2 Experimental Methods ........................................................................................... 63
3.2.1 Materials ....................................................................................................... 63
3.2.2 Characterization ............................................................................................ 64
3.2.3 Electrochemical System .............................................................................. 64
3.2.4 Synthesis of Ppy Nanowires ....................................................................... 65
3.2.5 Incorporation of Avidin and Streptavidin in Ppy Nanowires ..................... 65
3.2.6 Protein Quantification ................................................................................. 66
3.2.6.1 Accessible Active Sites ................................................................ 66
3.2.6.2 Total Active Sites ......................................................................... 67
3.2.6.3 Binding Kinetics of Biotin/Streptavidin ...................................... 68
3.3 Results and Discussion: Protein Incorporation into Ppy Segments ................... 68
3.3.1 Effect of Protein Charge ............................................................................ 70
3.3.2 Electropolymerization Potential ................................................................. 72
3.3.3 Electrochemical Method of Synthesis ......................................................... 72
3.3.4 Kinetics of Biotin/streptavidin Binding ....................................................... 73
3.4 Conclusions ............................................................................................................ 74
3.5 References .............................................................................................................. 76

Chapter 4: Polymer Nanowires with Catalytic Properties ....................................... 92
4.1 Introduction ............................................................................................................ 92
4.2 Experimental Methods ........................................................................................... 96
4.2.1 Materials ..................................................................................................... 96
4.2.2 Characterization .......................................................................................... 97
4.2.3 Electrochemical System ............................................................................ 97
4.2.4 Synthesis of Gold/polypyrrole Nanowires ............................................. 98
4.2.5 Catalase-modified Gold/polypyrrole Nanowires ........................................ 99
  4.2.5.1 Catalase Immobilization by Avidin-biotin Binding ......................... 99
  4.2.5.2 Catalase Immobilization by the Entrapment Method .................... 100
4.2.6 Synthesis of Polypyrrole Films and Catalase-modified Polypyrrole Films .......................................................... 100
4.2.7 Fabrication of Platinum Filled Gold/polypyrrole Nanowires .......... 101
  4.2.7.1 Incorporation of Pt Nanoparticles by Filtration of Nano-powder .... 101
  4.2.7.2 Incorporation of Pt Nanoparticles by Direct Reduction of PtCl$_2^{2-}$ 102
4.2.8 Oxygen Evolution Studies ................................................................. 102
4.2.9 Autonomous Movement Studies.................................................... 103
4.3 Results and Discussion ........................................................................ 104
  4.3.1 Gold/Ppy nanowires ..................................................................... 104
  4.3.2 Properties of Catalase-modified Gold/polypyrrole Nanowires ......... 104
    4.3.2.1 The Avidin-biotin Binding Affinity ..................................... 104
    4.3.2.2 The Entrapment Method ....................................................... 106
  4.3.3 Electrochemical Properties of Polypyrrole Films and Catalase-modified
    Polypyrrole Films .............................................................................. 110
  4.3.4 Properties of Platinum Filled Gold/polymer Nanowires ............... 113
4.4 Conclusion .......................................................................................... 115
4.5 References ......................................................................................... 116

Chapter 5: Nanowires Containing Electronic Polymer Junctions ............. 139
  5.1 Introduction ....................................................................................... 139
  5.2 Experimental Methods ................................................................. 145
    5.2.1 Materials .................................................................................. 145
    5.2.2 Characterization ...................................................................... 145
    5.2.3 Electrochemical System ........................................................... 146
    5.2.4 Synthesis of Electronic Polymer ............................................. 147
    5.2.5 Synthesis of Segmented Metal/EP/metal Nanowires ............... 149
LIST OF FIGURES

Figure 1.1: Library of nanowire structures developed in the Mallouk research group. (a) (W\textsubscript{12}O\textsubscript{41}/TiO\textsubscript{2})\textsubscript{3} nanotube filled with an Au wire for electronic applications, (b) 200 nm diameter Au/poly(ethylene dioxythiophene/gold wire for gas sensing applications, (c) 70 nm diameter gold wire with a 7 nm thick poly(pyrrole) coating, (d) 70 nm diameter wire containing a 16-mercaptohexanoic acid monolayer "stripe," (e) gold wire containing a photoconductive CdSe segment, (f) hollow tubule of 2 nm TiO\textsubscript{2} particles and poly(styrene sulfonate). ................................................................. 14

Figure 1.2: (a) Au/Ni/polypyrrole nanowire modified with avidin proteins at the polymer end, (b) Au/Ni/Au nanowires treated with ethylene dithiol. Some optical micrographs are embossed for clarity. ........................................................................................................ 15

Figure 1.3: Nano-scale sensor model: (a) segmented structure of a nanowire sensor; (b) integration of nanowire for sensor array. ........................................................................................................ 16

Figure 1.4: Chemical structures of the monomer precursors of the conducting polymer studied throughout this thesis, (a) pyrrole; (b) aniline; (c) ethylene-dioxythiophene....... 17

Figure 2.1: Diaz’s mechanism of pyrrole polymerization. ................................................ 39

Figure 2.2: Scanning electron micrograph of a gold nanowire array representing the empty volume that is filled with material during fabrication by template replication. Free-standing nanowires can be observed on the surface of the array. ................................. 40

Figure 2.3: Schematic diagram of the synthesis of segmented gold/polypyrrole nanowires by replication of alumina templates.............................................................. 41

Figure 2.4: Schematic diagram of the synthesis of segmented gold/polypyrrole/gold nanowires. ................................................................................................................. 42

Figure 2.5: Cyclic voltammogram of Ppy thin film grown from 0.1 M pyrrole in PBS solution, pH 7.4. Scan started at 0.0 V; first four scans are shown, recorded at 20 mV/s. ....................................................................................... 43

Figure 2.6: Cyclic voltammogram of Ppy Ecy nanowire growth from 0.2 M pyrrole in PBS solution, pH 7.4. Scan started at -0.2 V; first four scans are shown, recorded at 20 mV/s. ....................................................................................... 44

Figure 2.7: (a) Cross section of alumina membrane containing silver/gold/polypyrrole nanowires grown by fixing a counter electrode (Pt foil) parallel to the membrane; (b) Single Au/Ppy nanowire. ......................................................................................................................... 45
Figure 2.8: FE-SEM images of Ppy films synthesized at constant potential (0.75 V vs. SCE) at different pyrrole concentrations, (a) 0.1 M, (b) 0.3 M. ......................................................... 46

Figure 2.9: FE-SEM images of Ppy films synthesized by (a) constant potential (0.75 V), and (b) potential cycling (-0.2-0.8 V), from 0.2 M pyrrole in PBS. Scale bar 3 µm. ....... 47

Figure 2.10: Cyclic voltammogram of Eco nanowires and Ecy nanowires embedded in the alumina template recorded at 100 mV/s in PBS, pH 7.4. .............................................. 48

Figure 2.11: Electrochemical characteristics of alumina membranes containing Eco and Ecy nanowires cycled in PBS pH 7.4, and showing proportionality between peak current and scan rate characteristic of mass transport controlled processes. ....................................... 49

Figure 2.12: FE-SEM images of the difference in stiffness of (a) Eco nanowires, and (b) Ecy nanowires, scale bar 4.3 µm. FE-SEM images of the structure of Eco nanowires (c) external, and (d) internal, scale bar 300 nm. ................................................................. 50

Figure 2.13: TEM images of (a) Ecy nanowire, (b) Eco nanowire, scale bar 250 nm. (c) Sodium chloride growth inside an Eco nanowire, scale bar 200 nm. ................................. 51

Figure 2.14: Atomic force micrograph of a gold/polypyrrole nanowire (a) height, and (b) phase images. The red arrow indicates the Au/Ppy interface. .............................................. 52

Figure 2.15: (a) Growth rate of (■) Eco nanowires, and (◊) Ecy nanowires, grown from 0.2 M pyrrole, pH 7.4; (b) pH dependence of Ppy nanowire growth rate (µm/min.). Nanowires synthesized by: (□) E cycling (-0.2- 0.9 V, 20 mV/s), and (♦) constant E (0.75 V). ................................................................. 53

Figure 2.16: Growth rate as a function of oxidation potential for Eco nanowires grown at pH 5.5 (●), pH 7.4 (□), and pH 9 (∆). ................................................................. 54

Figure 2.17: (a) Cross section of alumina membrane containing gold/polypyrrole/gold nanowires, scale bar 2.5 µm; (b) Optical micrographs of an Au/Eco-Ppy/Au nanowire in bright-field mode, scale bar 1 µm. Diameter and total length are 314 nm and 4 µm, respectively. The inset shows a dark-field image of small aspect ratio Au/Eco-Ppy/Au nanowire, scale bar 4 µm. ................................................................. 55

Figure 2.18: (a) TEM image of Au/Eco-Ppy junction, scale bar 100 nm; the inset shows an ESEM micrograph of the same junctions, scale bar 1 µm; (b) FE-SEM micrograph of Au/Ppy junction after electroless deposition of second Au cap, scale bar 600 nm. (c) FE-SEM micrograph of an Au/Ppy/Au nanowire after electrochemical deposition of the second gold cap, scale bar 600 nm. ................................................................. 56

Figure 2.19: Cross section of alumina membrane containing scattered Au/Ppy and Au/Ppy/Au nanowires resulting from the direct electrodeposition of gold on Ppy segments, scale bar 2.5 µm. ................................................................. 57
Figure 2.20: Current-voltage characteristics of a single Au/Eco-Ppy/Au nanowire. The open circuit measurement shows a small current leakage in the device. Experimental details on methods for electrical characterization can be found in Chapter 5. .......................... 58

Figure 3.1: Schematic of (a) tetramer crystal structure of avidin and biotin chemical formula, (b) proteins in polymer thin films, (c) proposed protein-modified polymer nanowires. ......................................................................................................................... 79

Figure 3.2: Schematic of the proposed entrapment mechanism of proteins into nanowires during polymerization at pH 9. The incorporation of species by the doping mechanism proposes that negatively charge molecules will be attracted to the positively charged active polymer surface. ......................................................................................................................... 80

Figure 3.3: Schematic diagram of the methods used to quantify the amount of protein binding sites in Ppy nanowires. ......................................................................................................................... 81

Figure 3.4: Diagram of the step by step procedure employed to measure biotin binding kinetics to avidin or streptavidin-modified Ppy nanowires. ............................................................ 82

Figure 3.5: Detection of the presence of avidin in polypyrrole nanowires by UV-VIS spectroscopy at 280 nm, (a) spectra of 4 µM avidin solution in PBS, pH 7.4, (b) spectra of Ppy nanowires, (c) spectra of avidin-modified Ppy nanowires. All nanowires were suspended in PBS, pH 7.4 ........................................................................................................................................... 83

Figure 3.6: (a) Bright-field and (b) fluorescence images of Au/Ppy/Au nanowires containing avidin-RITC in the Ppy matrix, excited at 488 nm, recorded at λ > 512 nm..84

Figure 3.7: (a) Differential interference contrast (DIC), (b) false color fluorescence, (c) overlapped DIC and fluorescence images, and (d) close view of the overlap image of Au/Ppy nanowires containing avidin-Texas Red in the Ppy matrix, excited at 543 nm. Inset: SEM image of the internal structure of Eco nanowires. ................................................................. 85

Figure 3.8: Illustration of UV-VIS absorbance measurements for the determination of avidin and streptavidin in polypyrrole nanowires from absorption measurements of biotin-FITC conjugates by UV-VIS spectroscopy at 494 nm. ................................................................. 86

Figure 3.9: Binding sites in avidin/streptavidin-modified Ppy Eco nanowires as a function of pH. Total binding sites in streptavidin (●) and avidin (▲) modified nanowires; available binding sites after 10 hours in streptavidin (□), and avidin (○) modified nanowires. .................................................................................................................................................. 87

Figure 3.10: (a) Influence of polymerization potential on streptavidin incorporation in Ppy nanowires synthesized from 0.2 M pyrrole in PBS, at pH 5.5 (■), and pH 9 (●). (b) Streptavidin and avidin incorporated into nanowires synthesized at pH 9, as a function of constant oxidation potential. .............................................................................................................. 88
Figure 3.11: (a) Growth rate as a function of oxidation potential for Eco nanowires grown at pH 5.5 (●), pH 7.4 (□), and pH 9 (▲). (b) Binding sites in streptavidin-modified Ppy nanowires synthesized by a constant potential of 0.75 V, and by cycling the potential from -0.2 V to 0.9 V vs. SCE. Inset: growth rate of Eco nanowires (●), and Ecy nanowires (△). ................................................................. 89

Figure 3.12: Illustration of UV-VIS absorbance measurements of biotin-4-FITC remaining in supernatant solution for the study of binding kinetics of avidin or streptavidin modified nanowires ........................................................................................................ 90

Figure 3.13: Adsorption kinetics for biotin-FITC binding to Ppy nanowires. Streptavidin-Ppy nanowires (□), and unmodified nanowires (△) synthesized by constant E. Streptavidin-Ppy nanowires (●), and unmodified nanowires (▲) synthesized by E cycling. Lines are best fits to eq 1 ........................................................................................................ 91

Figure 4.1: Schematic diagram of (a) the interfacial tension model for Pt/Au propulsion mechanism, and (b) trajectory plots of three 2 µm long Pt/Au nanowires in 2.5% aqueous hydrogen peroxide. Reproduced with permission of Paxton et al.19 .............................................. 119

Figure 4.2: Schematic diagram of the synthesis of gold/polypyrrole nanowires and gold/catalase-modified nanowires by replication of alumina templates ......................... 120

Figure 4.3: Schematic diagram of the biochemical approach for the immobilization of catalase on Ppy nanowires using the avidin/biotin binding system ........................................ 121

Figure 4.4: (a) Optical image, and (b) TEM image of Au/Ppy nanowires ..................... 122

Figure 4.5: Optical micrographs of neutravidin-modified polystyrene beads reacted with the biotin/fluorescein-catalase; (a) differential interference contrast (DIC), (b) fluorescence image ........................................................................................................ 123

Figure 4.6: Optical and fluorescence images of Au/avidin-modified nanowires reacted with the biotin/fluorescein-catalase. Fluorescence of the entire nanowire revealed non-specific binding of the catalase conjugate to the gold surface ........................................ 124

Figure 4.7: Catalytic activity of catalase toward the decomposition of hydrogen peroxide to water and oxygen, analyzed by the amount of oxygen evolved in the system in free catalase (green circles), biotinylated catalase (blue squares), neutravidin-modified polystyrene beads reacted with biotinylated catalase (red triangles), avidin-modified nanowires reacted with biotinylated catalase (open squares), and catalase content on the supernatant solution from last rinsed after incubation of catalase complex with avidin-modified Ppy nanowires (yellow diamonds). ................................................................. 125

Figure 4.8: Picture of the western blot analysis of biotin/fluorescein-catalase conjugate. ........................................................................................................ 126
Figure 4.9: Rate of oxygen production by the catalytic activity of catalase embedded in Ppy segments of a Au/Ppy nanowire sample as released from the template (open squares), after exposure to 6 M urea for 1 hour (red diamonds), and after exposure to 6 M urea for 24 hours (blue squares).  

Figure 4.10: (a) CV of a gold disk electrode cycled in ferrocene solution between 0-400 mV vs. Ag/AgCl at 100 mV/s; bare gold surface (red), and Ppy film polymerized on a gold disk electrode surface (blue). (b) CV of a Ppy film polymerized on a gold disk electrode surface, cycled in PBS solution at 50 mV/s.  

Figure 4.11: (a) Ppy films synthesized from PBS solution and scanned immediately after polymerization (red-dotted line) and after soaking in PBS for 24 hours (red-solid line). (b) Ppy films synthesized from PBS solution and scanned immediately after polymerization (blue-dotted line) and after soaking in PBS for 24 hours (blue-solid line). All scans were performed at 20 mV/s.  

Figure 4.12: Ppy films polymerized from PBS (red) and PB (blue), and scanned in the corresponding buffer solution at 20 mV/s. (a) Scanned immediately after polymerization, (b) scanned after storing in corresponding buffer solution for 24 hours. (c) (a) and (b).  

Figure 4.13: (a) Ppy film (red-dotted line) and catalase-modified Ppy film (green-solid line) scanned immediately after polymerization. (b) Catalase-modified Ppy film scanned immediately after polymerization (green-solid line) compared to a Ppy film scanned after soaking in buffer for 24 hours. (c) (a) and (b). All scan were performed in PBS solutions at 20 mV/s.  

Figure 4.14: (a) Ppy film scanned immediately after polymerization (blue-dotted line); Ppy film after soaking in buffer for 24 hours (blue-solid line); catalase-modified Ppy film scanned immediately after polymerization (red-solid line); and catalase-modified Ppy film after soaking in buffer for 72 hours (red-dotted line). (b) Ppy film scanned after soaking in buffer for 24 hours (blue-solid line); and catalase-modified Ppy film scanned immediately after polymerization. All scan were performed in PB solutions at 20 mV/s.  

Figure 4.15: Ppy film scanned immediately after polymerization (blue) and catalase-modified Ppy film also scanned immediately after polymerization (red); (a) scan rate was 5 mV/s, (b) scan rate was 200 mV/s. All films were polymerized from PBS solutions.  

Figure 4.16: Catalase-modified Ppy film, scanned immediately after polymerization (blue), after exposure to 0.5 M NaOH for 1 hour (red), followed by soaking in PBS solution for 1 hour (green). The scanning rate was 20 mV/s.
Figure 4.17: TEM image of an Au/Pt-filled PEDOT nanowire fabricated by filtration of Pt nanopowder into the alumina template and subsequent polymerization of PEDOT to encapsulate the catalyst. Inset: TEM image of Pt nanoparticles used in this system. ...

Figure 4.18: Optical micrograph of a Ppy film (a) before, and (b) after doping and direct reduction of PtCl$_2^{2-}$. ................................................................. 136

Figure 4.19: TEM image of (a) a single Au/Ppy nanowire, and (b) multiple nanowires containing Pt nanoparticles at the polymer end by doping and direct reduction of PtCl$_2^{2-}$. ........................................................................................................ 137

Figure 4.20: Rate of oxygen evolution by the catalytic activity of Pt nanoparticles (red squares) electrochemically grown in Ppy segments of Au/Ppy nanowires as seen in Figure 4.19. Oxygen evolution rates for Au/catalase-modified Ppy nanowires (open squares), and Pt/Au nanowires (green triangles) is shown for comparison......... 138

Figure 5.1: Structure of (a) polarons and (b) bipolarons in PEDOT................................. 178

Figure 5.2: EDOT-based conducting polymer units developed by Swager and coworkers.\textsuperscript{21} (a) Tungsten (IV)-capped calixarene monomer (R= adamantyl group imparts specific recognition to xylene). (b) Conducting polymer sensitive to nitric oxide when M= cobalt.\textsuperscript{22} ........................................................................................................... 179

Figure 5.3: Schematic diagram of the synthesis of segmented gold/PEDOT/gold nanowires. ....................................................................................................................... 180

Figure 5.4: Schematic diagram of the photolithographic process for the fabrication of electrically isolated electrodes used in electric-field-assisted assembly of nanowires. (a) Au or TiPd bus bars are patterned on Si/SiO$_2$ substrates. (b) A dielectric layer (Si$_3$N$_4$) is deposited. (c) Au alignment electrodes are patterned onto the dielectric layer. (d) Capacitive coupling between bus bars and top electrodes induces nanowire alignment. (e) The aligned nanowires cut off capacitive coupling................................................... 181

Figure 5.5: New alignment electrode array containing 20 pairs of electrodes individually interconnected to large (100 $\mu$m x 100 $\mu$m) area pads for wire bonding. The bus bars (purple) are buried under a silicon nitride dielectric layer, inducing a capacitance effect on the top, alignment electrodes shown in green. The optical image shows large area pads designed for additional mechanical probing....................................................... 182

Figure 5.6: Summary of the nanowire integration process: (a) nanowires are aligned by capacitive coupling between the bus bars and the alignment electrode that produces an electric field; (b) alignment chips are wire-bonded to a 40-pin DIP package; (c) pins in the DIP package are connected to external instrumentation........................................... 183

Figure 5.7: (a) 100 $\mu$m x 100 $\mu$m interdigitated array (IDA) structures. (b) 100 $\mu$m x 100 $\mu$m IDA floating structure on microheaters........................................................... 184
**Figure 5.8:** Gold nanowires aligned on Au-IDA by cycling the potential ±10 V........ 185

**Figure 5.9:** (a) SEM of polyaniline on bi-metallic IDA. (b) polyaniline on Pt-IDA at an early stage (5 cycles). (c) SEM and optical image of esmeraldine salt form of polyaniline on Pt-IDA after 20 cycles. ................................................................. 186

**Figure 5.10:** (a) Polyaniline film exposed to HCl of different pH values. (b) Dedoped polyaniline film exposed to 2 M HCl. ................................................................. 187

**Figure 5.11:** Electrical response of polyaniline on IDA exposed to 1% NH₃ at the chemical sensing facilities at PSU (courtesy of Ms. Yanyan Cao). ................................. 188

**Figure 5.12:** CV of the synthesis of polyaniline nanowires from 0.1 M aniline in 2 M HCl, and potential sweeps between 0 to 0.85 V vs. SCE at 50 mV/s............................... 189

**Figure 5.13:** TEM image of Au/polyaniline nanowires. The red box shows the polyaniline-Au junction. ......................................................................................... 190

**Figure 5.14:** CV of the synthesis of PEDOT nanowires from 0.05 M EDOT, 0.1 M TBA PF₆ in methylene chloride; from 0 to 2500 mV vs. Ag wire, 100 mV/s......................... 191

**Figure 5.15:** TEM micrographs of PEDOT nanowire showing apparent open ends. .... 192

**Figure 5.16:** Typical electron micrographs of electrodeposited gold/PEDOT:TBAPF₆ junctions......................................................................................................................... 193

**Figure 5.17:** TEM images showing electrodeposited gold (dark color) onto PEDOT (light color) segments (a) near the gold-polymer junction of the nanowires, and (b) at the polymer end. ......................................................................................................................... 194

**Figure 5.18:** TEM images showing the electroless deposition of gold on PEDOT ends at (a) early stage (~3 hours), and (b) after 24 hours. (c) Gold growth inside PEDOT. ...... 195

**Figure 5.19:** TEM images of an improved (a) PEDOT-electroless gold junction, and (b) solid electroless gold segment. .............................................................................. 196

**Figure 5.20:** (a) Typical I-V characteristics of Au/PEDOT (red) and Au/PEDOT/Au (black) nanowires. (b) I-V of Au/PEDOT/Au nanowire (black) showing a slight deviation from ohmic (red) behavior. ................................................................. 197

**Figure 5.21:** I-V of Au/PEDOT/Au nanowire before (black) and after (red) exposure to aqueous hydrazine for 30 minutes. The inset shows an expanded portion of the I-V characteristics from 0-3 V of Au/PEDOT/Au nanowire after exposure to hydrazine.... 198
Figure 5.22: Electrical response of a single Au/PEDOT:PSS/Au nanowire exposed to water vapor in the chemical sensing facilities at NIST: (a) 25 ppm, (b) 250 ppm. 199

Figure 5.23: Electrical response of a single Au/PEDOT:PSS/Au nanowire exposed to 1% NH$_3$ at the chemical sensing facilities at PSU. 200
LIST OF TABLES

Table 5.1: Conducting polymers of high value in the semiconductor and electronic markets.

Table 5.2: Number of Au nanowires aligned in a single IDA vs. resistance before and after electroplating gold on the electrodes.

Table 5.3: Resistance of single polyaniline nanowires aligned in IDA.
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1.1 Brief Overview of Nanoparticle Technology

An evident driving force in the research and development of new materials aims at the miniaturization of devices down to the ultimate limits. Nano-scale particles such as nanowires, nanotubes, and nanocrystals have been established as smart building blocks for emerging trends in nanostructured electronic and sensing devices. Many unique and fascinating properties have already been demonstrated, such as superior mechanical toughness, higher luminescence efficiency, and lower lasing threshold. Because of their very high aspect ratio, high electronic conductivity, and small size, nanowires and nanotubes offer the potential of high sensitivity, low power operation, and massive redundancy in nanosensor arrays. In addition, homogeneous nanowires and nanowire networks have been previously used as chemical sensors, field effect transistors, photodetectors, light emitting diodes and lasers, and logic gates.

The Mallouk research group in the chemistry department at the Pennsylvania State University (PSU) has developed nanowire conductors, rectifiers, switches, photoconductors, and sensors (see Figure 1.1), among others. Mallouk and Kovtyukhova have designed complex nanowire structures containing diode heterojunctions and coaxially-gated in-wire thin film transistors, a step forward to the potential of constructing nanowire-based logic circuits. The photoconducting properties of nanowires containing polycrystalline semiconductor segments have been demonstrated and recent work on single crystal semiconductor nanowires synthesized by laser-assisted vapor-
liquid-solid growth mechanism exhibited superior performance.\textsuperscript{12} Lastly, nanowires containing molecular junctions,\textsuperscript{13} and conducting polymers\textsuperscript{14} are other examples of the extensive library of anisotropic particles and materials investigated by the Mallouk research group and by the scientific community.\textsuperscript{15}

1.2 Overview of Fabrication Techniques for Nanowire Assemblies

The development of nanostructured materials has been the focus of research and development over the past several decades due to the enhancement in electronic, optical, and magnetic properties that occurs when electrons are strongly confined in one or two dimensions. So far, there are four general approaches that have been employed to fabricate nanowire/nanorod structures: nanolithography-based methods, solution-based methods, vapor deposition, and template fabrication.

The nanolithography-based method is a widely used technique in the fabrication of 1D nanostructures\textsuperscript{16} that employs advanced lithographic techniques such as electron beam lithography, x-ray lithography, or projection lithography with deposition and plasma etching processes. This method is slow and expensive and currently is not suitable for large scale fabrication of 1D nanostructures.

The solution-based approach to the synthesis of nanowires employs controlled wet chemical reactions.\textsuperscript{17} In general, a solvent with a certain precursor, catalyst, and a template agent (e.g., surfactants) are reacted to form nanostructures that precipitate out of solution. The drawbacks to this process are that it requires a detailed understanding of the chemical reaction and growth mechanism, and the products are usually polydisperse and not pure. Further, it is currently beyond the scope of this technique to make complex
heterostructures such as segmented nanowires that contain specific sequences of active components.

Vapor-based methods consist of vapor transport or vapor reactions at a suitable temperature and pressure. One example of a vapor-based method is vapor-liquid-solid (VLS) growth. This method employs a catalyst to promote anisotropic crystal growth, and a large number of materials have been grown into nanowire form based on this process. A specific catalyst must be chosen for each material, and the growth temperature is usually relatively high.

The template method uses anisotropic nanoporous materials, such as anodized alumina, track-etched polycarbonate membranes, or block copolymer membranes to serve as the host. The pores in the template may be filled by any deposition method (e.g., electrochemical reaction, chemical reaction, sol-gel, vapor deposition, etc.) to generate the desired 1D nanowires. This can be considered an alternative to conventional lithographic methods. Historically, the method was introduced when Possin et al. prepared different metallic nanowires with diameters as small as 40 nanometers in pores of track-etched mica. Refined later by Giordano and Moskovits, it has become increasingly used, especially for the preparation of monodisperse nanowires and nanotubes.

Anodized alumina is a key template material for the fabrication of nanomaterials because it can provide large area, nanometer size structures with high aspect ratios, and can be fabricated inexpensively and reproducibly. Unlike track-etch membranes, anodized alumina has pores with little or no tilt with respect to the surface normal resulting in a non-connecting, straight, and cylindrical pore structure as the one shown in
Appendix A. These are some of the characteristics that make anodized alumina the template of choice for the fabrication of the different types of composite nanowires discussed throughout this thesis.

1.3 Nanowire Assemblies

In comparison to spherical particles, anisotropic particles offer additional degrees of freedom and greater challenges in self-assembly due to their inherent shape. Chemical interactions and electric fields are increasingly studied as techniques for the controlled self-assembly of nanowires from colloidal suspensions. Mallouk and Natan’s work on the orthogonal self assembly of molecules on colloidal gold/platinum nanowires was the first demonstration of the ability to chemically pattern the surface of bi-metallic nanowires based on surface chemical reactivity with nano-scale control. These results stimulated a number of publications by other groups and furthermore, motivated the use of very sophisticated molecules to modify the surface of nanowires for different applications.

The original research objective of this thesis was to create a complex structure of segmented gold/nickel/polypyrrole (Ppy) modified with avidin proteins at the polymer end (see Figure 1.2a). The nanowires would be reacted with complementary strands of thiolated oligonucleotides type A and biotinylated oligonucleotide type B that would bind to the gold ends and the polymer ends, respectively. The result would be particles with programmed assembly properties by means of DNA hybridization. Initial experiments on Au/Ni/Au nanowires treated with ethylene dithiol were intended to confirm the poor affinity of the native surface of the nickel segment to thiol-containing molecules, and
therefore, achieve chemical modification of only the gold segments of nanowires in a single step. Nanowires containing ethylene dithiol gold ends assembled in solution in a variety of linear geometries and shapes just at the gold tips as shown in Figure 1.2b. This result corroborated that ethylene dithiol did not bind to the nickel segments in the nanowires. Next, the development of Ppy nanowire segments modified with avidin proteins evolved dramatically into a much larger research program on a new class of conducting polymer nanowire for sensor applications and other systems. Nevertheless, colleagues at PSU continue to explore DNA technology methods for the directed assembly of nanowires onto surfaces and assemblies.\textsuperscript{25}

The electric-field-assisted alignment of nanowires has been developed and continuously refined by scientists and engineers at PSU.\textsuperscript{26} The method is extensively used to position individual nanowires of any conductive material between two microelectrodes and facilitate electrical characterization and device fabrication. Experimental details on the electric-field assisted alignment method are described in Chapter 5 since it is employed for the electrical characterization of nanowires containing polymer junctions.

1.4 Nanowire Sensor Model

Research on hybrid organic/inorganic materials is an expanding area in materials science that constitutes a class of advanced composite materials with unusual properties. In general, the formation of hybrids between conducting polymers and inorganic solids aims to create composite materials with synergetic or complementary behavior between the polymer and the inorganic portion. Additionally, the ability to synthesized nanowires with different segments along the length of the nanostructure provides the opportunity to
introduce multiple chemical functionalities by exploiting the selective properties of the different segments. Figure 1.3 depicts the nano-scale sensor model that motivated the development of the many kinds of metal/conducting polymer nanocomposite structures described in this thesis.

Gold nanowires containing a conducting polymer segment were designed and fabricated to meet the requirements of a particular sensor or assembly application. The materials integrated into these nanocomposite structures were selected for their electrical properties, sensory response, biocompatibility and stability. Gold segments impart a stable, ohmic, low resistance electrical contact to the nanowire structure for integration into lithographically designed electrode surfaces, making possible the study of electrical properties of individual nanowires. The inherent properties of the conducting polymer segments offer a wide range of electrical and chemical properties that can be tailored to applications such as chemical or biological sensors, and electronic devices. There are some key factors to consider when fabricating the nanowire structure presented in Figure 1.3:

- material’s properties must be compatible with an electrical transduction mechanism
- there must be good adhesion between the interfaces of the various materials to ensure mechanical stability as well as intimate electrical contact between segments.
- the fabrication conditions of the different materials (metal, polymer, and biomolecules) in the composite structure must not alter the properties of the other materials already present in the structure.
Meeting the above criteria can be a challenge in the development of any composite structure. Therefore, this thesis provides many synthetic methodologies and strategies for the development of nanowire structures with applications in the area of biosensing, chemical sensing and more.

1.4.1 Brief Overview of Conducting Polymers

Since the first report of electrical conductivity in a conjugated polymer (polyacetylene) made in 1977, the possibility of combining in these materials the chemical properties of organic polymers and the electronic properties of semiconductors had been the driving force of thousands of academic and industrial researchers. Conducting polymers can be synthesized by chemical or electrochemical oxidation of their monomer units. The latter is generally preferred because it provides a better control of thickness and morphology, and cleaner polymers when compared to chemical oxidation. The conducting polymers employed in the present work: Ppy, poly(aniline) (PANI), and poly(ethylene dioxythiophene) (PEDOT) (see Figure 1.4); were electrochemically synthesized. Electrochemical synthesis was preferred in many ways due to relatively easy controllability of experimental conditions, which affect the polymer stability as well as chemical, electrical, and electrochemical properties.

1.5 Objectives and Organization of the Thesis

This thesis is composed of five chapters that document the research activities performed in the Chemical Science and Technology Laboratory at the National Institute
of Standards and Technology (NIST) (Gaithersburg, MD) and the chemistry department at PSU. This work focused on the development of composite nanowire structures toward the development of nano-scale sensors arrays and other assemblies.

Chapter 2 presents a comprehensive study of the experimental conditions and properties of composite gold/Ppy nanowires synthesized from phosphate-buffered saline (PBS) solutions. Ppy was synthesized from a buffer solution to promote a biocompatible environment for the subsequent incorporation of proteins into the polymer segments as described in Chapter 3 and 4.

Chapter 3 investigates the introduction of the proteins avidin and streptavidin into the Ppy nanowires by entrapment during Ppy polymerization. The effects of pH and electrodeposition potential on the incorporation of proteins were studied to establish a viable approach to control protein incorporation. The high specificity of the association between these proteins and biotin was used to monitor protein incorporation and accessibility in the conducting polymer segments of the nanowires as a function of the conditions of synthesis.

Chapter 4 employs the methodology described in Chapter 3 to incorporate the enzyme oxido reductase (catalase) into Ppy nanowires. The introduction of the enzyme into the nanowire structure imparts Ppy with catalytic power to decompose hydrogen peroxide from within the polymer matrix. Additionally, the enzyme showed chemical stability to high concentrations of urea, a denaturing agent, demonstrating that the entrapment method locks the proteins into an active conformation. Such properties can be employed in the development of nano-scale electrochemical biosensors and delivery systems.
Finally, Chapter 5 describes the synthetic strategies employed in the fabrication of composite nanowires containing electronic polymer junctions, nanowire self-assembly and packaging, and the gas dilution system developed for sensor measurements. This chapter is a brief summary of the initial phase of a major research program intended to develop high-density, on-chip electronic sensor arrays with the capacity to resolve trace analytes in complex environmental mixtures. This research is currently led by Ms. Yanyan Cao in the Mallouk research group.
1.5 References


Figure 1.1: Library of nanowire structures developed in the Mallouk research group. (a) \((W_{12}O_{41}/TiO_2)_3\) nanotube filled with an Au wire for electronic applications, (b) 200 nm diameter Au/poly(ethylene dioxythiophene/gold wire for gas sensing applications, (c) 70 nm diameter gold wire with a 7 nm thick poly(pyrrole) coating, (d) 70 nm diameter wire containing a 16-mercaptohexanoic acid monolayer "stripe," (e) gold wire containing a photoconductive CdSe segment, (f) hollow tubule of 2 nm TiO_2 particles and poly(styrene sulfonate).
Figure 1.2: (a) Au/Ni/polypyrrole nanowire modified with avidin proteins at the polymer end, (b) Au/Ni/Au nanowires treated with ethylene dithiol. Some optical micrographs are embossed for clarity.
Figure 1.3: Nano-scale sensor model: (a) nanowire sensor segmented structure; (b) integration of nanowire for sensor array.
Figure 1.4: Chemical structures of the monomer precursors of the conducting polymer studied throughout this thesis, (a) pyrrole; (b) aniline; (c) ethylene dioxythiophene.
Chapter 2

Fabrication and Characterization of Conducting Polypyrrole Nanowires

2.1 Introduction

2.1.1 Properties of polypyrrole

Polypyrrole (Ppy) is the most extensively studied conducting polymer over the past twenty years. Its interesting properties as well as its environmental stability make Ppy suitable for use in electronics, batteries, electrochromic devices, selective membranes, (bio) sensors, capacitors, drug delivery systems, among others.\(^1\) Conducting Ppy can be prepared by chemical or electrochemical polymerization methods. In the chemical polymerization process, monomers are oxidized by oxidizing agents with or without catalysts to produce conducting polymers.\(^2\) One advantage of chemical synthesis is that it offers mass production at reasonable cost; however, the presence of catalyst materials contaminates the final product and requires additional processing steps to purify the system. On the other hand, the electrochemical method involves the direct formation of conducting polypyrrole with better control of thickness and morphology. Figure 2.1 depicts the most cited polymerization mechanism for Ppy, the Diaz’s mechanism.\(^3\) During the initial oxidation, a radical cation of the monomer is formed and reacts with other monomers to form oligomers and subsequently the polymer. The conjugation in the polymer backbone lowers its oxidation potential compared to that of the monomer. Consequently, synthesis and doping of the polymer occur simultaneously and anions are incorporated into the polymer to ensure electrical neutrality.
The quality and properties of electrochemically synthesized Ppy films depend on many different factors, *e.g.*, doping anion, electrochemical method of synthesis, selected solvent, monomer concentration, and electrode substrate.\textsuperscript{3,4} Since pyrrole has a relatively low oxidation potential,\textsuperscript{5} electropolymerization can be carried out in aqueous electrolytes, something that is difficult or impossible for other heterocyclic monomers such as thiophene and benzene. This chapter discusses the electrochemical synthesis of polypyrrole films and nanowires from PBS solutions, and the most favorable conditions of polymer growth for (bio) sensing applications.\textsuperscript{6}

### 2.1.2 Polypyrrole Nanowires and Nanotubes

Ppy is an interesting material for incorporation into nanowire-based sensors and nanostructures because of its high environmental stability, electronic conductivity, ion exchange capacity, and biocompatibility.\textsuperscript{7,8} These properties have made Ppy a popular constituent of planar electrochemical biosensors.\textsuperscript{9,10}

Martin et al.\textsuperscript{11} have investigated the electrochemical and chemical template-synthesis of Ppy within the pores of polycarbonate membranes. They prepared Ppy nanotubules of varying diameters and found higher conductivity than in Ppy thin films, which was attributed to alignment of polymer chains along the pore axis. Consequently, the study of structure-property relationships of conducting polymer nanostructures has become a subject of interest.\textsuperscript{12} Other strategies for the fabrication of conducting polymer nanowires have entailed electrochemical synthesis on grafted insulating polymeric layers or on self-assembled monolayers.\textsuperscript{13} While this method cleverly uses surface chemistry to direct the formation of nanowires, it lacks control over nanowire length, directionality,
and dispersity. Recently, the self-assembly of Au/Ppy and Au/Ppy/Au nanowires into three-dimensional vesicle-like structures was reported. These nanowires were synthesized using a similar approach to that described in this chapter. However, the methods described here were modified to provide a biocompatible environment for incorporation of proteins (see Chapter 3).

This chapter gives an account of the synthesis and characterization of segmented Au/(Ppy)/Au nanowires (300nm in diameter and a few microns long) as the first step towards the development of nano-scale biosensors and assemblies (further studies in Chapter 3). Some of the parameters (pH, monomer concentration, and electrochemical method of growth) that affect the growth of Ppy nanowires in a biocompatible environment were studied. These nanowires were made using anodic alumina templates in PBS by two different electrochemical methods: constant potential and potential cycling. Previous studies showed that the choice of electrochemical method has an influence on the morphology, appearance and adhesion of Ppy films. In fact, this work shows that the choice of electrochemical method also leads to differences in the kinetic and mechanical properties of the nanowires that are relevant to their use in sensors and self-assembling structures.
2.2 Experimental Methods

2.2.1 Materials

Anodic alumina membranes (Whatman Inc., NJ) containing cylindrical pores ca. 0.3 \( \mu \text{m} \) in diameter were employed as the templates for nanowire growth. Pyrrole (98+%), \( \text{SnCl}_2 \) (99+%), \( \text{AgNO}_3 \) (100%), \( \text{Na}_2\text{SO}_3 \) (99.5%), formaldehyde (37%), Methanol (99.96%), \( \text{Na}_2\text{CO}_3 \) (99.5%), and mercaptoacetic acid (98+%), were obtained from Aldrich. Aqueous ammonia (30%) and trifluoroacetic acid (neat) were obtained from J.T. Baker and Supelco, respectively. Silver (1025) and Au (Orotemp 24) electroplating solutions, and \( \text{Na}_3\text{Au(SO}_3\text{)}_2 \) (Oromerse Part B, 8 x \( 10^{-3} \) M) were obtained from Technic Inc., Cranston, RI. Ultrapure water (18 M\( \Omega \)-cm) was used for the preparation of all solutions and for rinsing. PBS solutions were prepared with 0.1 M \( \text{NaCl} \) (99.0%, Sigma), 0.003 M \( \text{KCl} \) (99.7%, J.T.Baker), and 0.002 M total phosphate concentration using potassium dihydrogen phosphate (99+, Sigma) and disodium hydrogen phosphate (100%, J.T.Baker) to obtain pH values of 5.5, 7.4, and 8.8. HCl (37%, Aldrich) or \( \text{NaOH} \) (97+, Aldrich) was added to obtain the lower and higher pH buffers. Silicon substrates and were purchased from Addison Engineering, Inc., San Jose, CA.

2.2.2 Characterization

Optical and fluorescence imaging was achieved with an Axiotech 100 reflected-light microscope (Carl Zeiss, NY) equipped with a CoolSNAP HQ camera (Roper Scientific, Inc., AZ). Scanning electron microscope (SEM) images were obtained with a FEI-Phillips-Electrosan model 2020 environmental scanning electron microscope (E-
SEM), and a field-emission scanning electron microscope (Hitachi S-4100 cold-cathode FE-SEM). Transmission electron microscope (TEM) images were obtained with a JEOL 1200 EXII at 80 kV accelerating voltage. Electrochemical properties were studied using the electrochemical system described in the next section.

2.2.3 Electrochemical System

All electrochemical experiments were performed using a potentiostat/galvanostat (Princeton Applied Research, model 263A) in a one-compartment, three-electrode cell at room temperature (Appendix A). A platinum foil (2 cm²) and a saturated calomel electrode (SCE) served as the counter electrode and reference electrode, respectively. A thin layer (150 nm) of sacrificial Ag was deposited on the branched side of the anodic alumina membranes (Appendix A) by thermal evaporation to serve as the working electrode. Electrochemical deposition occurred inside the alumina membranes producing straight, monodisperse composite nanowires of desired lengths. Polymer films were grown onto gold-coated silicon substrates also assembled in a three-electrode cell configuration.

2.2.4 Synthesis of Conducting Polypyrrole Films

Conducting polypyrrole films were grown onto silicon substrates coated with a 150 nm Au layer by anodic oxidation (electropolymerization). A series of experiments were performed to determine the most favorable conditions for Ppy growth in PBS solutions, including the effect of pH, monomer concentration, and electrochemical method of polymerization. Ppy films were grown by applying a constant potential to the
working electrode (Eco) or by cycling the applied potential (Ecy). Eco Ppy films were
grown at 0.75 V vs. SCE, from 0.2 M pyrrole solutions in 150 mM PBS at pH 5.5, 7.4,
and 8.8, unless otherwise stated. Ecy-Ppy films were grown by potential sweeps between
-0.2 V to 0.9 V vs. SCE at 20 mV/s. Monomer solutions were bubbled with nitrogen
immediately prior to use, and the electrochemical cell was protected from light to prevent
monomer oxidation in solution.

2.2.5 Synthesis of Polypyrrole Nanowires

Polypyrrole nanowires were synthesized by a template replication method using
the electrochemical system described in section 2.2.3. The templates used throughout
this work were alumina membranes characterized by having a branched side and an un-
branched side that result from the anodization process employed to produce cylindrical
pores. A sacrificial silver layer was evaporated on the branched side of 60 µm think
anodic alumina membranes containing cylindrical pores ca. 0.3 µm in diameter to serve
as the working electrode. Approximately 3 µm of additional sacrificial silver was
electrodeposited into the alumina membrane to fill the branched section of the template
and prevent branched nanowire structures. Figure 2.2 shows an array of gold nanowires
obtained by electrodeposition of gold into the alumina template, fixing the template to a
glass slide via epoxy, and finally dissolution of the alumina template. The array
represents the porous volume that when filled with material yields the nanowire
structures. Figure 2.3 depicts the fabrication of segmented gold/conducting polymer
nanowires inside the templates.
In addition to the experimental insight on polypyrrole electro-polymerization from PBS solutions gained during the preparation of conducting polymer films, additional experiments were performed to study the growth of Ppy nanowires further. Solutions ranging from 0.05 M to 1 M pyrrole, pH 7.4, were used to investigate the effect of monomer concentration on nanowire growth rate and homogeneity throughout the porous alumina membrane. As in the case of Ppy films, Ppy nanowires were grown from 0.2 M pyrrole solutions in 150 mM PBS at pH 5.5, 7.4, and 8.8 for all other experiments. All solutions were bubbled with nitrogen immediately prior to use, and the electrochemical cell was protected from light to prevent monomer oxidation in solution. Although Ppy can be generated galvanostatically (0.48 mA/cm² current density), growth proved to be susceptible to gradients in the potential across the membrane, resulting in polydisperse wires. This problem was minimized by fixing the distance from the counter electrode to the alumina membrane at 2 cm for all experiments, and by polymerizing at a constant potential (constant E) of +0.75 V or by E cycling between -0.2 V and +0.9 V vs. SCE at 20 mV/s. (Nanowires synthesized by constant E and E cycling are designated henceforth as Eco nanowires and Ecy nanowires, respectively)

The alumina membranes were subsequently treated with 25% (v/v) HNO₃ and 3 M sodium hydroxide to obtain freestanding nanowires. Nanowires were rinsed three times with water and three times with methanol or ethanol, and then re-suspended in methanol or ethanol.
2.2.6 Synthesis of Segmented Metal/polymer/metal Nanowires

Figure 2.4 illustrates the synthesis of segmented gold/polypyrrole/gold nanowires. Electro-deposition of Au was carried out at a constant potential of −0.9 V vs. SCE using a commercial Au plating solution containing approximately 6.9% potassium aurocyanide (KAu(CN)₂). Au containing membranes were soaked in pyrrole solutions for 10 minutes prior to polymerization. Ppy segments were electrodeposited as described in the previous section. The membrane containing Au/Ppy was then immersed in 10 mM mercaptoacetic acid solution in methanol for 12 hours. The second Au segment was grown by a slightly modified version of the electroless deposition method described by Menon et al.¹⁸ The membranes were successively reacted at 25°C with 0.025 M SnCl₂ and 0.07 M trifluoroacetic acid in 50:50 methanol/water (1hr), 0.03 M AgNO₃ in 0.3 M aqueous ammonia (10 min.), and a solution containing 0.008 M Na₃Au(SO₄)₂/ 0.129 M Na₂SO₃/ 0.625 M HCHO adjusted to pH 10 (12 hrs), then rinsed with copious amounts of methanol and water. The electrolessly-deposited gold layers were elongated by electrochemical deposition of gold at a constant potential of −0.9 V vs. SCE.

2.3 Results and Discussion

2.3.1 Polypyrrole Films and Nanowires: Monomer Concentration and Solvents

Figure 2.5 shows the cyclic voltammogram (CV) of a Ppy thin film grown from 0.1 M pyrrole in PBS solution at pH 7.4. Oxidation of the monomer begins at 0.6 V vs. SCE showing a characteristic current increase as the applied potential is increased. Two small peaks can be observed at -0.08 and 0.02 V vs. SCE, corresponding to the reduction and oxidation of the already deposited Ppy layers. An important characteristic of Ppy is
that its oxidation potential is lower than that of the monomer (pyrrole) and thus the polymer is simultaneously oxidized during polymerization.\(^{19}\)

Figure 2.6 shows the cyclic voltammogram for the growth of Ppy nanowires inside alumina membranes by E cycling in PBS, pH 7.4, recorded at a scan rate of 20 mV/s. A cross section of an alumina membrane containing the resulting gold/polypyrrole nanowires is shown in Figure 2.7. As in the case of Ppy films, the steep rise in anodic current at 0.6-0.7 V corresponds to oxidation of the pyrrole monomer, whereas the cathodic and anodic peaks at -0.20 V and +0.05 V correspond to reduction and oxidation of the electrodeposited polymer. Because oxidation of the monomer occurs at a much higher potential than that of the polymer, side reactions including nucleophilic attack and overoxidation, and/or crosslinking of the polymer should be expected. These reactions are potentially important, particularly in the incorporation of proteins that contain nucleophilic functional groups, and in the derivitization of the polymer with thiol-containing molecules.

The effect of monomer concentration on polypyrrole film morphology was determined by varying the concentration of pyrrole monomer during polymerization at constant potential (0.75 V vs. SCE) and is shown in Figure 2.8. Throughout this chapter, SEM studies on electropolymerized Ppy films show globular particle aggregates in accordance with the literature reports. Films deposited from 0.1 M pyrrole concentration revealed smaller size and nearly spherical grains than those deposited from 0.3 M pyrrole solution (Figure 2.8b). The differences in structure suggest that higher monomer concentration will lead to very uncontrollable growth of protruding polymer grains.
To study the effect of monomer concentration in nanowires, the concentration of pyrrole was varied from 0.05 M to 1 M to determine the effect of monomer concentration on Ppy Eco nanowire growth rate. Growth rate increased with increasing monomer concentration; however, very polydisperse nanowire lengths were observed at low or high concentrations. It was found that a concentration of 0.2 M gave good control [±4%] over nanowire length.

Aprotic solvents, such as acetonitrile, are known to be best for Ppy synthesis. Unsworth et al. have shown that the adsorption of oxygen gas formed during water oxidation is a source of surface defects in Ppy films regardless of any initial deoxygenation step. Pores created in this process may be desirable in terms of the permeability of analyte molecules; however, structural defects could deteriorate the electronic integrity of the nanowires.

The rationale behind the electrochemical synthesis of Ppy is that when a Ppy monolayer is deposited on an electrode surface, it will be quickly covered by a second monolayer. Simultaneously, the already deposited layers are subject to further oxidation (oxidative degradation). The rate of electrochemical oxidation of the deposited layers is dependent on the transport of counter-anions through the layers. Previous chronoamperometric studies on Ppy films in aqueous solutions considered the effects of chloride and phosphate as counter ions. Electrodes prepared in chloride solution were in the electronically conductive oxidized state, and oxidation of monomers in solution could take place. In contrast, those electrodes prepared in phosphate solution were in the nonconductive neutral state. In our studies, both anions were present in solution with a chloride concentration ten-fold higher than phosphate. Under these conditions, the effect
of chloride ions appears to dominate, and both Ppy Eco and Ecy nanowires exhibited electroactivity (see Figure 2.10).

The effect of solutions such as aqueous sodium hydroxide, ethanol and methanol on the overall properties and performance of Ppy nanowires was an initial concern. These solutions were employed to release and to clean the nanowires from the alumina template into solution. Previous work on the exposure of Ppy films (prepared from aqueous phosphate buffer for amperometric biosensing applications) to NaOH/methanolic solutions address some of the chemical overoxidation effects. X-ray photoelectron spectroscopy (XPS) studies on overoxidized Ppy films confirmed hydroxyl functionalities on the top layers and methoxy groups in the bulk region. XPS also revealed that methoxylation and hydroxylation occurred mainly in the beta-position to pyrrole nitrogen and the double bond conjugation was not extensively affected. Accordingly, Ppy maintained remarkable electronic conductivity even after exposure to NaOH and methanol. These findings supported the experimental methods employed here for the release of Ppy nanowires as part of the processing steps towards the development of amperometric nano-scale biosensors. The electrical characteristics of these nanowires are discussed in section 2.3.5.

2.3.2 Polypyrrole Nanowires: Electrochemical Method of Synthesis

As Zhou and Heinze pointed out in their work, the method of electrochemical synthesis controls the structural form of electrodeposited Ppy films. To investigate the structural differences of Ppy grown by different electrochemical methods, films were electrochemically grown from solutions containing 0.2 M pyrrole in PBS onto 200-nm
thick gold-covered silicon substrates by constant potential (+0.75 V vs. SCE) and cycling the potential (-0.2 V to +0.9 V vs. SCE, 20 mV/s). Electron micrographs of these films are shown in Figure 2.9. Films obtained by E cycling were shiny black, with smaller grains whereas larger “cauliflower” structures were formed with constant potential deposition. These results are in agreement with the work of Otero and DeLarreta, who studied the effects of constant current, potential and alternating polarizations on polypyrrole structure. Since both polypyrrole films and nanowires are grown using the same parameters, it should be expected that nanowires also exhibit differences in their structure by varying the electrochemical method of synthesis.

The CV of membranes containing Eco and Ecy nanowires were recorded from PBS solutions, pH 7.4, in the absence of monomer and are compared in Figure 2.10. The CV of Eco nanowires showed well defined peaks characteristic of oxidation/reduction processes of polypyrrole films. Two anodic peaks at -0.3 V and 0.35 V are associated with two oxidation processes that are normally observed in the presence of chloride ions. A corresponding cathodic peak at -0.3 V was also observed. On the other hand, Ecy nanowires showed smaller sharp anodic and cathodic peaks (indicative of slow charge transfer diffusion or slow counterion diffusion in the polymer) superimposed on a background of broader anodic and cathodic waves. The latter is indicative of slow electron transfer kinetics at the Ppy/solution interface. These characteristics are explained in later sections and may be associated with the differences in microstructure that results from the growth of Ppy by the two different methods.

The membranes containing Eco and Ecy nanowires were cycled in PBS solution, pH 7.4, at various scan rates from 5 to 200 mV/s, and the relationship between cathodic
peak current and scan rate is plotted in Figure 2.11. For both types of nanowires the cathodic peak currents are proportional to the square root of the scan rates, characteristics of processes that are limited by the rate of mass transport.\textsuperscript{26}

A variation in the stiffness of nanowires synthesized by the two electrochemical methods was apparent when lengths exceeded 4\(\mu\)m as illustrated in Figure 2.12a,b. Ecy nanowires were straight even at lengths over 10 \(\mu\)m; in contrast, long Eco nanowires were flexible and had spaghetti-like shapes after release from the alumina template. Figures 2.12c and 2.12d show the external and internal morphology of a Ppy Eco nanowire, respectively. The internal structure was revealed by agitating the nanowire solution to produce openings along the wire, and was found to be quite porous compared to the external surface. On the other hand, the stiffness of Ecy nanowires made it impossible to produce open nanowires for imaging by simple agitation.

TEM images of Eco and Ecy nanowires are shown in Figure 2.13. These images confirm the synthesis of Ppy nanowires versus nanotubes. Structural differences between the two types of nanowires were not obvious (Figure 2.13a and 2.13b), however, TEM images like that in Figure 2.13c were only found in some Eco nanowires. The dark internal features in Figure 2.13c are consistent with the porosity observed in Figure 2.12d. Such features could be due to the formation of salt crystals from pockets of PBS trapped in the Eco nanowires. This characteristic was not observed in Ecy nanowires, suggesting the absence of free volume inside Ecy nanowires. Although both electrochemical methods yielded nanowires with smooth, featureless surfaces (consistent with polymer wetting of the pore walls of the template), the differences in their electrochemical behavior (Figure 2.10) and their rates of analyte adsorption (see below)
are likely to result from differences in their internal structure. Atomic force microscopy (AFM) was used to verify the apparent smoothness of the surface of Ppy nanowires as it appears in the TEM images in Figure 2.13. An AFM micrograph of a gold/Ppy nanowire is shown in Figure 2.14. The top right segment of the nanowire in the image corresponds to part of the Ppy segment and the bottom left region to the gold portion. The height image reveals slight a difference between both regions, showing a very smooth polymer region and a grainier gold segment. These differences are more apparent in the phase image in Figure 2.14b. The very grainy phase image is probably due to the polycrystalline nature of the gold segments. These AFM data and all other surface characterization techniques confirm that the surface of Ppy segments are atomically smooth.

The growth rates of Eco nanowires and Ecy nanowires are given in Figure 2.15a. The growth of nanowires exceeding lengths over ten micrometers is irregular for both electrochemical methods of synthesis and is more evident for nanowires grown at constant potential.

It should be noted that all experimental parameters have a significant impact on the properties of the resulting polymer and that changing a single variable (such as acidity) may induce a more profound change in the polymer structure and properties than a change in the applied potential.

2.3.3 Polypyrrole Nanowires: pH Dependence

Eco nanowires and Ecy nanowires were grown for various lengths of time and number of cycles, respectively. Even though the monomer oxidation potential is
independent of the pH, pH has an influence on the reactivity and stability of polypyrrole. Faster growth is observed at lower pH, as revealed in Figure 2.15b. This can be explained by the fact that an acidic environment favors the transformation of pyrrole into a radical cation, hence increasing the number of nucleation sites during polymerization. Moreover, it is known that overoxidation of Ppy occurs more readily at higher pH, and that significant nucleophilic attack occurs in aqueous solutions at pH ≥ 4.5. However, to obtain the most favorable growth conditions for the incorporation of proteins and other biomolecules into Ppy, it is necessary to find a compromise between biocompatible pH conditions and polymer stability (see Chapter 3).

2.3.4 Polypyrrole Nanowires: Oxidation Potential

Nanowires were grown at constant potentials of 0.6, 0.75, 0.9 V vs. SCE from 0.2 M pyrrole solutions at pH 5.5, 7.4, and 9, and the effects on growth rate are illustrated in Figure 2.16. Growth rate increased with increasing applied potential but once again became inconsistent due to fast, inhomogeneous polymerization. This is the result of unpredictable nucleation and fast growth at the solution/polymer interface. A potential of 0.75V vs. SCE, was generally used to synthesize nanowires by constant potential since it provided some control over nanowire lengths.

Parameters such as monomer concentration and oxidation potential can yield exceedingly high polymerization rates resulting in polydisperse samples. In nanosensor applications requiring self-assembly, monodisperse particles are necessary and these parameters must be adjusted to yield controllable growth.
2.3.5 Segmented Au/Polypyrrole/Au Nanowires

Eco nanowires were selected for the synthesis of segmented Au/Ppy/Au nanowires since their physical, electrochemical, and kinetic properties proved to be more suitable for biosensing applications (see chapter 3) than Eco nanowires. Au/Ppy/Au nanowires were made by successive electrodeposition of Au, electropolymerization of Ppy at constant E, and electroless gold deposition (Figure 2.17). Ppy segments were capped with gold at each end in order to make possible their integration into lithographically designed electrodes by electrofluidic alignment and to impart low resistance contacts in subsequent electrical measurements during future operation (see Chapter 5). Good physical contact and sharp junctions between the Ppy segment and the top gold segments of the nanowires could be achieved by following an electroless plating procedure in which the exposed Ppy was first exposed to mercaptoacetic acid. The purpose of this step was the nucleophilic addition of thiol groups to the Ppy surface in order to create reactive carboxylate functionality for electroless deposition of gold.\(^{31}\) Sn(II) was complexed by the carboxylate groups by immersion of the membrane in SnCl\(_2\) solution, and Ag nanoparticles were then deposited via reaction of Sn(II) with Ag(I). After thorough rinsing with methanol and water, Au was then electrolessly deposited from a solution containing Na\(_3\)Au(SO\(_3\))\(_2\), Na\(_2\)SO\(_3\), and formaldehyde.

The initial Au/Ppy junction produced by the electrochemical deposition of Au, followed by electropolymerization of Eco-Ppy, yielded smooth and strong junctions (Figure 2.18a). Au/Eco-Ppy nanowires were capped by two different methods: electroless deposition of Au as described above, and direct electrochemical deposition of Au onto Ppy. Ppy/Au junctions obtained by these two methods were quite different in
structure, as seen in Figures 2.18b and 2.18c. The electroless gold top contacts did not appear to penetrate the Ppy segments significantly (Figure 2.18b). On the other hand, electrodeposited gold permeated the Ppy segments of Eco nanowires leading to very poorly defined junctions and in many cases physical contact (electrical short) between the top and bottom Au segments (Figure 2.18c, and 2.19). We explain this phenomenon in terms of electrostatic interactions between ionic species and the polymer matrix. The electrodeposited Ppy is cationic and should exchange anions with contacting solutions. In the case of the electroless Au plating bath, the anion in highest concentration is \( \text{SO}_3^{2-} \), which is likely to compete effectively with \([\text{Au(SO}_3]_2\text{]}^{3-}\) for ion exchange sites in the polymer. The Au electroplating solution contains both \([\text{Au(CN)}_2]^{-}\) and \(\text{CN}^{-}\) anions. It is possible that \([\text{Au(CN)}_2]^{-}\) ions exchange into the polymer and that this leads to deposition of metallic gold throughout the polymer matrix rather than just at the tip. Figure 2.19 shows another consequence of the direct electrodeposition of Au over the surface of Ppy segments. Scattered Au/Ppy and Au/Ppy/Au nanowires can be seen from a cross section of an alumina membrane. This picture reveals that in some instances, Au electrodeposition does not occur on the surface of Ppy segments. The logical cause of this observation is that charge is not transferred through those areas of the membrane, suggesting that the polymer is highly insulating at those points. Moreover, an insulating state might be instantaneously induced in some areas of the membrane when applying the negative potential required for metal electrodeposition. In conclusion, an electroless deposition method is required for capping the Ppy segments. Finally, the top Au cap was extended by further electrochemical deposition of Au to produce segmented nanowires as shown in Figure 2.17.
The current-voltage characteristics of a single Au/Eco-Ppy/Au nanowire are presented in Figure 2.20. (The experimental details of the method for characterizing the electrical properties of individual nanowires are explained in Chapter 5.) These electrical measurements show that nanowires synthesized from PBS solutions have a very high resistance (GΩ). These results are not surprising since it is known that the most favorable conditions to promote biomolecule stability (for future biosensor applications) are the most unfavorable ones for the synthesis of conducting polymers. Although some current can be measured, it may be necessary to perform further polymer processing to increase Ppy conductivity to practical levels.

2.4 Conclusions

The electrochemical replication of porous alumina membranes provided a straightforward route to free-standing nanowires of controlled length. Because these nanowires are designed to be employed together with biological systems, the conditions of polymer deposition are constrained to aqueous buffers that are bio-compatible. Although overoxidation of Ppy occurs in these buffers, the polymer exhibits electroactivity. The electrodeposition method (constant potential or potential cycling) has a marked effect on the morphology and electrochemical properties of the conducting polymer segments. In general, the most favorable conditions for the growth of bio-compatible Ppy nanowires are 0.75V vs. SCE from 0.2 M pyrrole solutions in 150mM PBS at pH 7.4. These conditions provide good control of nanowire growth, and yield nanowires with an internal porosity suitable for the entrapment of a variety of species.
2.5 References


10 Cosnier, S., Biosensors & Bioelectronics 1999, 14, 443.


22 Zhao, S.; Luong, J.H.T. Electroanalysis 1995, 7, 633


Figure 2.1: Diaz’s mechanism of pyrrole polymerization.
Figure 2.2: Scanning electron micrograph of a gold nanowire array representing the empty volume that is filled with material during fabrication by template replication. Free-standing nanowires can be observed on the surface of the array.
3 µm of silver are electroplated at 0.5 mA/cm².

1 µm of gold is electroplated at 0.5 mA/cm².

Polypyrrole is electropolymerized at 0.75 V vs. SCE.

Silver is dissolved in 25% v/v HNO₃. The alumina template is dissolved in 0.5 M NaOH.

Figure 2.3: Schematic diagram of the synthesis of segmented gold/polypyrrole nanowires by replication of alumina templates.
Au/Ppy nanowires are modified with mercaptoacetic acid solution.

Gold is electrolessly deposited.

Gold layer is elongated by electrochemical deposition of gold.

Silver is dissolved in 25% v/v HNO₃. The alumina template is dissolved in 0.5 M NaOH.

Figure 2.4: Schematic diagram of the synthesis of segmented gold/polypyrrole/gold nanowires.
Figure 2.5: Cyclic voltammogram of Ppy thin film grown from 0.1 M pyrrole in PBS solution, pH 7.4. Scan started at 0.0 V; first four scans are shown, recorded at 20 mV/s.
Figure 2.6: Cyclic voltammogram of Ppy Ecy nanowire grown from 0.2 M pyrrole in PBS solution, pH 7.4. Scan started at -0.2 V; first four scans are shown, recorded at 20 mV/s.
Figure 2.7: (a) Cross section of alumina membrane containing silver/gold/polypyrrole nanowires grown by fixing a counter electrode (Pt foil) parallel to the membrane; (b) Single Au/Ppy nanowire.
Figure 2.8: FE-SEM images of Ppy films synthesized at constant potential (0.75 V vs. SCE) at different pyrrole concentrations, (a) 0.1 M, (b) 0.3 M.
Figure 2.9: FE-SEM images of Ppy films synthesized by (a) constant potential (0.75 V), and (b) potential cycling (-0.2-0.8 V), from 0.2 M pyrrole in PBS. Scale bar 3 µm.
Figure 2.10: Cyclic voltammogram of Eco nanowires and Ecy nanowires embedded in the alumina template recorded at 100 mV/s in PBS, pH 7.4.
Figure 2.11: Electrochemical characteristics of alumina membranes containing Eco and Ecy nanowires cycled in PBS pH 7.4, and showing proportionality between peak current and scan rate characteristic of mass transport controlled processes.

![Graph showing the relationship between peak current and the square root of scan rate. The graph includes two lines, one for E Cycling and another for E Constant, indicating a linear relationship with increasing scan rate.]
Figure 2.12: FE-SEM images of the difference in stiffness of (a) Eco nanowires, and (b) Ecy nanowires, scale bar 4.3 µm. FE-SEM images of the structure of Eco nanowires (c) external, and (d) internal, scale bar 300 nm.
Figure 2.13: TEM images of (a) Ecy-nanowire, (b) Eco-nanowire, scale bar 250 nm. (c) Sodium chloride growth inside an Eco-nanowire, scale bar 200 nm.
Figure 2.14: Atomic force micrograph of a gold/polypyrrole nanowire (a) height, and (b) phase images. The red arrow indicates the Au/Ppy interface.
Figure 2.15: (a) Growth rate of (■) Eco nanowires, and (◊) Ecy nanowires, grown from 0.2 M pyrrole, pH 7.4; (b) pH dependence of Ppy nanowire growth rate (μm/min.). Nanowires synthesized by: (□) E cycling (-0.2- 0.9 V, 20 mV/s), and (♦) constant E (0.75 V).
Figure 2.16: Growth rate as a function of oxidation potential for Eco nanowires grown at pH 5.5 (●), pH 7.4 (□), and pH 9 (▲).
Figure 2.17: (a) Cross section of alumina membrane containing gold/polypyrrole/gold nanowires, scale bar 2.5 µm; (b) Optical micrographs of an Au/Eco-Ppy/Au nanowire in bright-field mode, scale bar 1µm. Diameter and total length are 314 nm and 4 µm, respectively. The inset shows a dark-field image of small aspect ratio Au/Eco-Ppy/Au nanowire, scale bar 4 µm.
Figure 2.18: (a) TEM image of Au/Eco-Ppy junction, scale bar 100 nm; the inset shows an ESEM micrograph of the same junctions, scale bar 1 µm; (b) FE-SEM micrograph of Au/Ppy junction after electroless deposition of second Au cap, scale bar 600 nm. (c) FE-SEM micrograph of an Au/Ppy/Au nanowire after electrochemical deposition of the second gold cap, scale bar 600 nm.
Figure 2.19: Cross section of alumina membrane containing scattered Au/Ppy and Au/Ppy/Au nanowires resulting from the direct electrodeposition of gold on Ppy segments, scale bar 2.5 µm.
Figure 2.20: Current-voltage characteristics of a single Au/Eco-Ppy/Au nanowire. The open circuit measurement shows a small current leakage in the device. Experimental details on methods for electrical characterization can be found in Chapter 5.
Chapter 3

Protein-modified Polypyrrole Nanowires

3.1 Introduction

Nanowires and nanotubes modified with different proteins\textsuperscript{1,2} and oligonucleotides\textsuperscript{3,4} have recently been studied for the electronic detection of species of biological relevance. Lieber and coworkers\textsuperscript{5} have made biosensors by aligning amine-modified silicon nanowires onto electrodes patterned on a silicon wafer to create a nanowire field effect transistor (FET). These derivatized nanowires were modified with biotin to produce sensors for streptavidin or an anti-biotin antibody. Similarly, biosensors based on individual carbon nanotube FETs have recently been made by covalently linking proteins to the surface of nanotubes in order to impart chemical selectivity. While these devices have shown excellent sensitivity, they rely on post-synthesis covalent modification of nanowires and nanotubes that can be difficult to produce, purify, and manipulate. Nanowires and nanotubes modified with biological molecules are also interesting building blocks for self-assembling nanostructures because very specific biomolecular interactions can be used to program their assembly.\textsuperscript{6}

Although several strategies for the synthesis of Ppy nanowires and nanotubes have been reported,\textsuperscript{7,8} their applications as nanoscale biosensors are only beginning to be explored. Gao et al.\textsuperscript{9} recently developed a glucose sensor based on an array of carbon nanotubes coated with Ppy-glucose oxidase. The array constituted a planar electrode with an extraordinary increase in surface area resulting from the polymer-coated nanotubes.
Two methods have been reported for the immobilization of proteins in conducting polymer films. In one method, the polymer is electrochemically grown from a buffer solution containing the proteins, which are encapsulated in the growing polymer film.\textsuperscript{10} In this method, the activity of the protein may be affected by the presence of monomer.

In another method, monomer (e.g., pyrrole, aniline) is polymerized in an acidic solution to form a strong cohesive film on a platinum electrode; and then proteins are immobilized based on the doping principle of conducting polymers.\textsuperscript{11} The incorporation of species by the doping mechanism presumes that negatively charged molecules will be intercalated into the positively charged polymer matrix to attain charge neutrality. Previous work on the immobilization of galactose oxidase in polyaniline films by the doping principle showed that the enzyme electrode had a good bioelectrochemical response to galactose.\textsuperscript{12} However, the galactose oxidase in polypyrrole film electrode showed a very small response compared to its polyaniline analog.\textsuperscript{13} The reason for the low response is that smaller amounts of protein were incorporated into the Ppy electrode. The particles of the Ppy electrode were arranged tightly on the film surface with spacing of about 50 Å, which is smaller than the diameter of an enzyme (100-1000 Å), and this obstructed the pathway of protein into the matrix. Thus, the work described in this chapter employed the entrapment method to ensure maximum incorporation of the proteins; avidin and streptavidin, into polymer nanoparticles. Nevertheless, the doping principle was investigated by studying the effect of pH (related to the protein net charge) on incorporation.

Avidin and streptavidin are biotin-binding proteins, and some of the most utilized biomolecules in biotechnology, nanotechnology, and molecular biology in addition to
DNA. Avidin is a glycoprotein found in egg white and tissues of birds, reptiles and amphibians, with molecular mass of 68,000 daltons and isoelectric point of 10. Streptavidin is isolated from *Streptomyces avidinii*, and unlike avidin, it has no carbohydrate and has an acidic isoelectric point of 5-6. The affinity of the molecule biotin (see Figure 3.1a) to both avidin and streptavidin is extremely strong ($K_a = 1 \times 10^{15} \text{M}^{-1}$), which is practically irreversible and similar in its stability to a covalent bond. The strong interaction is due to the shape specificity of the biotin binding pocket, which allows the formation of multiple hydrogen bonds and van der Waals interactions. This binding is stable over a wide range of pH values and temperatures, and it has been used for a wide range of applications including immunoassays, cytochemistry, protein purification, and diagnostics. Both avidin and streptavidin are homotetramers (see Figure 3.1a) and have four biotin-binding sites, with pairs on opposite sides of the proteins, making them suitable for use as intermediate building blocks in molecular assemblies. In very recent years, the avidin-biotin system has been applied to the immobilization of bio-active molecules on electrode surfaces for the purpose of fabricating bioanalytical sensors. In addition, biotin-modified antibodies, proteins, oligonucleotides, nanoparticles, and fluorescent molecules are commercially available or can be easily prepared, making this molecular system accessible to many kinds of scientific studies. One major difference between these proteins is that avidin carries a positive charge at neutral pH whereas streptavidin is nearly neutral. The similarities in molecular structure and these differences in net charge were exploited in this chapter to investigate the effect of charge on the amount of protein content in Ppy nanowires, and the proposed entrapment mechanism.
This chapter reports the synthesis of segmented Au/(Ppy)/Au nanowires (300 nm in diameter and a few microns long), as described in Chapter 2, loaded with proteins in the polymer segment as the first step towards the development of nanoscale biosensors and assemblies. Avidin and streptavidin were introduced into the nanowires by entrapment during Ppy polymerization,\textsuperscript{10,21} and the effects of pH, method of polymerization, and the polymerization potential were studied. The high affinity between these proteins and biotin was used to characterize protein incorporation and accessibility in Ppy nanowires and to determine the synthesis conditions for maximum protein loading, and performance.
3.2 Experimental Methods

3.2.1 Materials

All electrochemical experiments were performed with a potentiostat/galvanostat (Princeton Applied Research, model 263A) in a one-compartment cell at room temperature. A Pt foil (2 cm$^2$) and a Saturated Calomel Electrode (SCE) served as the counter electrode and reference electrode, respectively. Anodic alumina membranes (Whatman Inc., NJ) containing cylindrical pores ca. 0.3 µm in diameter were employed as the templates for nanowire growth. A thin layer (150 nm) of sacrificial Ag was deposited on the branched side of the membranes by thermal evaporation to serve as the working electrode. Pyrrole (98+%), 4-nitrophenyl phosphate disodium salt hexahydrate(97+%), and bovine serum albumin (BSA, 96+%) were obtained from Sigma. Methanol (99.96%), Na$_2$CO$_3$ (99.5%), and mercaptoacetic acid (98+%), were obtained from Aldrich. Silver (1025) and Au (Orotemp 24) electroplating solution were obtained from Technic Inc., Cranston, RI. Avidin, streptavidin, avidin-rhodamine isothiocyanate (RITC), avidin-fluorescein isothiocyanate (FITC), avidin-Texas Red, streptavidin-FITC, biotin, and biotin-FITC were obtained from Molecular Probes (Eugene, OR). Biotinylated alkaline phosphatase was purchased from Pierce (Rockford, IL). Ultrapure water (18 MΩ-cm) was used for the preparation of all solutions and for rinsing. PBS solutions were prepared with 0.1 M NaCl (99.0%, Sigma), 0.003 M KCl (99.7%, J.T.Baker), and 0.002 M total phosphate concentration using potassium dihydrogen phosphate (99+%%, Sigma) and disodium hydrogen phosphate (100%, J.T.Baker) to obtain pH values of 5.5, 7.4, and 8.8. HCl (37%, Aldrich) or NaOH (97+%%, Aldrich) was added to obtain the lower and higher pH buffers.
3.2.2 Characterization

Scanning electron microscopy (SEM) images were obtained with a FEI-Phillips-Electrosan model 2020 environmental scanning electron microscope (E-SEM), or a field-emission scanning electron microscope (Hitachi S-4100 cold-cathode FE-SEM). Optical imaging was achieved with an Axiotech 100 reflected-light microscope (Carl Zeiss, NY) equipped with a CoolSNAP HQ camera (Roper Scientific, Inc., AZ) and a tungsten lamp. Fluorescence images were acquired using the Axiotech 100 reflected-light microscope equipped with a CoolSNAP HQ camera and an air-cooled argon-ion laser system (643 series, Melles Griot, CA) in dark field mode. In addition, an Olympus IX70 inverted confocal microscope equipped with fluorescence burner, argon (488nm), and HeNe (543nm) laser lines, and a halogen light source was used to acquire high resolution fluorescence images. Absorbance measurements were performed with a fiber optic spectrometer (Model SD2000, Ocean Optics, Inc., Dunedin, FL) equipped with tungsten-halogen and deuterium lamps (Model DT 1000 CE, Analytical Instrument System, Inc., Flemington, NJ).

3.2.3 Electrochemical System

All electrochemical experiments were performed using a potentiostat/galvanostat (Princeton Applied Research, model 263A) in a one-compartment, three-electrode cell at room temperature (Appendix A). A platinum foil (2 cm²) and a saturated calomel electrode (SCE) served as the counter electrode and reference electrode, respectively. A thin layer (150 nm) of sacrificial Ag was deposited on the branched side of the anodic
alumina membranes by thermal evaporation to serve as the working electrode to synthesize nanowires.

3.2.4 Synthesis of Ppy Nanowires

The synthesis and characterization of Ppy nanowires is described in detail in Chapter 2. Chapter 2 concluded that the electrodeposition method (constant potential or potential cycling) employed in the synthesis of polypyrrole has a marked effect on the morphology and electrochemical properties not only of conducting Ppy films, but also of Ppy nanowires. Therefore, Chapter 3 describes the incorporation of proteins in nanowires synthesized by both constant potential (Eco nanowires) and by cycling the potential (Ecy nanowires) to determine the most suitable system for biosensing applications.

3.2.5 Incorporation of Avidin and Streptavidin in Ppy Nanowires

Immobilization of proteins into the Ppy matrix involved the physical entrapment of avidin, streptavidin or their fluorescent conjugates during polymerization. Figure 3.2 illustrates the concept of biomolecule immobilization in electropolymerized electrode surfaces. When an appropriate potential is applied to the working electrode immersed in a solution containing both protein and monomer molecules, the proteins present in the vicinity of the electrode surface are thus incorporated into the growing polymer matrix. The same principle should apply to the growth of protein-modified polypyrrole nanowires.
Protein-modified Ppy wires were grown from solutions containing 0.2 M pyrrole and 10 mg/mL of protein (avidin or streptavidin) in PBS (pH 5.5, 7.4, and 9) at constant E of 0.75 V or by E cycling between -0.2 V and 0.9 V vs. SCE at 20 mV/s. The nanowires were rinsed with PBS solution of the same pH as the polymerization solution and resuspended in PBS. A 1mL sample of 10 mg/mL Avidin-RITC in PBS was reacted with 300 µL of 10 mM D-bitoin in PBS prior to incorporation, and was used to monitor the uptake of avidin in the Ppy segments of the nanowires by means of fluorescence imaging.

3.2.6 Protein Quantification

All nanowires used in this study were 320±10 nm in diameter and 2±0.2 µm long as determined by FE-SEM. Figure 3.3 illustrates the two methods used for the quantification of protein in Ppy nanowires.

3.2.6.1 Accessible Active Sites

Binding assays were performed on protein-modified nanowires using standard biotin-FITC solutions (5-10 µM) in PBS at 25º, pH 7.4, for 10 hours under constant agitation (in some cases binding assays were performed at different lengths of time). The amount of avidin or streptavidin entrapped in the nanowires was quantified by measuring the change in absorbance of the biotin-FITC stock solution at 494 nm before and after performing binding assays. A 1mL aliquot of stock solution containing (10^6 particles/mL) protein-modified nanowires was incubated with 0.5 mL of 10 µM biotin-FITC in PBS. The nanowire solution was centrifuged, and nanowires were washed and
resuspended in PBS (pH 7.4) for subsequent experiments. Absorbance measurements were performed on the supernatant to determine the amount of biotin-FITC remaining in solution. The amount of biotin-FITC bound to the nanowires was calculated from the difference between the concentrations in supernatant and the biotin stock solution, and converted to the number of protein binding sites in the nanowires.

### 3.2.6.2 Total Active Sites

A modification of an indirect enzyme-linked binding assay method\(^2\) was employed to estimate the total number of biotin binding sites per nanowire. The method is based on the fact that the reaction between an enzyme-biotin conjugate and avidin reduces the catalytic activity of the enzyme by altering its tertiary structure and/or because of steric effects. Avidin and streptavidin standard solutions were incubated with an excess amount of alkaline phosphatase-biotin conjugate and p-nitro-phenyl phosphate. The activity of biotinylated alkaline phosphatase was monitored from the absorbance at 405 nm of the \(p\)-nitrophenol that is generated by the active enzyme. Aliquots of avidin or streptavidin were added to the binding assay, and calibration curves were generated by plotting the change in absorbance per unit time vs. protein concentration. To quantify the number of binding sites in the protein-modified nanowires, the nanowires were sonicated to rupture the Ppy matrix and to access buried protein molecules. As a control experiment, avidin and streptavidin standard solutions were sonicated for 1 minute. This assay showed that 1-min sonication had little effect on the number of biotin binding sites in avidin or streptavidin samples. A 1mL of sample solution containing \(10^7\) protein-modified nanowires/mL was resuspended in an assay buffer containing 0.05 M sodium.
carbonate (pH 9.4), 0.1 M NaCl, 0.01% (w/v) NaN₃, 0.01% (w/v) gelatin and 0.2% (w/v) BSA. The samples were sonicated for 10 seconds, the solutions were filtered with a PTFE filter (pore diameter ca. 0.2 µm), and incubated with excess biotinylated alkaline phosphatase for 10 min. A 50 µL aliquot of p-nitro-phenyl phosphate (20 mM) was added, and the absorbance of the samples was recorded over a 1-min period.

3.2.6.3 Binding Kinetics of Biotin/Streptavidin

Figure 3.4 illustrates the step by step analytical procedure used to study the kinetics of binding between streptavidin-modified nanowires and biotin suspended in solution. A suspension of 6.7 x 10⁷ streptavidin-modified Ppy Eco nanowires/mL prepared in PBS, pH 9, was reacted with 15 mM biotin-FITC at room temperature under constant stirring. Aliquots of 1mL were drawn out periodically and reacted with excess unlabeled biotin in order to desorb any nonspecifically bound biotin-FITC molecules. The solutions were centrifuged for 20 minutes and the amount of unreacted biotin-FITC was determined from absorbance measurements of the supernatant at 494 nm. The amount of bound biotin-FITC was plotted as a function of incubation time. A suspension of 3.5 x 10⁷ streptavidin-modified Ppy Ecy nanowires/mL prepared in PBS, pH 9, was studied in the same way.

3.3 Results and Discussion: Protein Incorporation into Ppy Segments

Avidin and streptavidin were successfully incorporated into Ppy nanowire segments by physical entrapment during electropolymerization. Several optical methods were utilized to probe the incorporation of protein into Ppy nanowires. Measuring the
ultraviolet light absorbed by aromatic residues in proteins at 280 nm is the simplest, most straightforward method for estimating protein content. Figure 3.5 shows UV-VIS spectra that demonstrate the presence of the protein avidin in Ppy nanowires. A peak at 280 nm is evident for avidin-modified nanowires corresponding to protein trapped in Ppy.

Figure 3.6 shows a bright-field and corresponding fluorescence image of segmented Au/Eco-Ppy/Au nanowires modified with avidin-RITC excited at 488 nm acquired with the reflected-light microscope set up described in section 3.2.2. Fluorescence was detected in the Ppy portion of the nanowires, verifying the incorporation of proteins into Ppy nanowires by the entrapment method. Figure 3.7 shows differential interference contrast (DIC) and fluorescence images of Au/Ppy nanowires containing avidin-Texas Red acquired in a confocal microscope (see section 3.2.2), excited at 543 nm. Long Ppy segments were utilized for imaging purposes only. The confocal system provided better resolution and a close view to the fluorescent areas corresponding to the protein’s location in the polymer segment. Fluorescence occurred throughout the entire polymer segment showing that the protein concentration remained constant near the working electrode (polymerization area). Although fluorescence was seen throughout the entire length of the polymer segment, an irregular pattern can be seen in Figure 3.7d. This fluorescence irregularity suggests that proteins may be trapped in pockets within the Ppy matrix generated during electropolymerization similar to the structure seen in the inset (also Figure 2.13d). These internal compartments in polymer nanowires could be exploited for a variety of applications including drug delivery.
Chapter 4 exploits the application of Ppy nanowires for the entrapment and protection of the enzyme oxido reductase.

3.3.1 Effect of Protein Charge

In previous work, the enzyme xanthine oxidase was immobilized into polyaniline films using a doping method that proposed that the enzyme carrying a negative charge (at pH values greater than its isoelectric point) was doped into the film during the polymer oxidation. Additional work on the electrochemical immobilization of the enzyme into polypyrrole films based on the doping principle further strengthen the theory of enzyme doping of conducting polymer films given that the polymer nanostructure permits the diffusion of proteins.

In this chapter, the effect of avidin and streptavidin net charge on incorporation into the Ppy nanowires was studied by varying the pH of the solutions during polymerization. Avidin and streptavidin are biotin-binding proteins with very different isoelectric points (10 and 5 to 6, respectively) due to differences in their amino acid sequences, thus serving as a good model for the study of the effect of pH. In these studies, the net charges in avidin were positive and the net charges in streptavidin were negative (except at the low end of the pH range studied, pH 5.5, where streptavidin is nearly neutral).

Biotin-FITC was reacted with nanowires containing the different proteins, and the amount of protein incorporated in the nanowires was indirectly determined from the concentration of biotin-FITC reacted by absorption measurements of the FITC moiety in conjugates by UV-VIS spectroscopy at 494 nm (see Figure 3.8).
Based on the work on xanthine oxidase, great differences were expected in the amounts of positively-charge avidin and negatively-charge streptavidin incorporated into polypyrrole nanowires. Nevertheless, Figure 3.9 shows that the amount of protein incorporated in Ppy nanowires does not have a significant pH dependence, even in the case of streptavidin near its isoelectric pH. Also, the difference between avidin and streptavidin incorporation is small at all values of pH. These results suggest that charge compensation of the cationic Ppy polymer is not the primary driving force for protein incorporation. Nucleophilic addition of reactive protein functional groups (NH$_2$, SH) at the surface of the growing Ppy segment$^{24}$ and hydrophobic protein-polypyrrole interactions$^{25}$ are other possible mechanisms for protein incorporation into the polymer. Two different protein assays (see Figure 3.3) were used to obtain the data shown in Figure 3.9. The close correspondence between the total number of biotin binding sites and the number of binding sites that are accessible after 10 hours in the intact wires indicates that essentially all the protein molecules incorporated are active (i.e., can bind biotin) given a sufficiently long equilibration time.

Figure 3.10a shows the amount of streptavidin incorporated in Ppy nanowires as a function of electropolymerization potential in acidic (pH 5.5) and basic (pH 9) buffered solutions. In basic environment, streptavidin is a negatively charge protein, and a higher content of streptavidin was observed in general at the basic pH than at the lower pH value. This trend is consistent with the doping mechanism of conducting polymers employed by Mu and coworkers, nevertheless, nanowires synthesized at pH 5.5 contained a substantial amount of protein, supporting the conclusion that charges are not the predominant force for protein incorporation into nanowires.
3.3.2 Electropolymerization Potential

Eco nanowires modified with avidin and streptavidin were grown at different potentials at pH 9. Figure 3.10b illustrates the amount of avidin and streptavidin incorporated in the nanowires as a function of polymerization potential. Nanowires synthesized at constant 0.6 V contained more avidin and streptavidin than nanowires synthesized at constant 0.9 V. The growth rate of Ppy nanowires as a function of oxidation potential is described in Chapter 2, and is directly related to the amount of protein incorporated in the nanowires (Figure 3.11b, also shown in Figure 2.17). Polymerization at 0.9 V occurred relatively quickly and may exceed the diffusion rate of proteins into the nanopores, yielding lower amounts of protein in the nanowires. At 0.6 V, polymerization is exceedingly slow and proteins are in equilibrium concentrations inside the nanopores. Furthermore, Figure 3.10 shows that streptavidin incorporation is maximized at lower potentials where growth is slow.

3.3.3 Electrochemical Method of Synthesis

Section 2.3.2 explains the effect of the electrochemical method of synthesis on the electrochemical properties and structural properties of nanowires. It was concluded that nanowires synthesized by different electrochemical methods have very different properties due to the differences in their growth mechanisms. Therefore, it is to be expected that the electrochemical method of synthesis should have an effect on protein incorporation and this was briefly studied. Figure 3.11b shows the number of binding sites in streptavidin-modified Ppy nanowires synthesized from 0.2 M pyrrole in PBS, pH
9, by applying a constant potential of 0.75 V, and by cycling the potential from -0.2 V to 0.9 V vs. SCE. Streptavidin was chosen over avidin to obtain nanowires with the highest protein content possible and to attribute any difference solely to growth rate and growth mechanism. Ecy nanowires revealed a higher streptavidin content than Eco nanowires, attributable to the difference in their growth rate (inset: Figure 3.11b and Figure 2.16). Naturally, Ecy nanowires have a lower growth rate than Eco nanowires due to the time required to complete a cycle and the smaller amount of the deposited material that results from the deposition/stripping growth mechanism induced by the cycling of the potential. The additional time required achieving growth on Ecy nanowires and the cycling mechanism resulted in higher protein content in the nanowires.

3.3.4 Kinetics of Biotin/Streptavidin Binding

The amount of biotin-FITC bound or absorbed to streptavidin-Ppy nanowires ($X_t$) was measured as a function of time (see Figure 3.12) for nanowires made by constant E and E cycling, as shown in Figure 3.13. Here, saturation with biotin-FITC ($X_s$) was reached in approximately 100 minutes and 300 minutes for Eco and Ecy nanowires, respectively. Biotin-FITC was also adsorbed onto Eco nanowires that contained no streptavidin, but the saturation coverage was only about 1/10 that of the streptavidin-modified nanowires. The kinetics of biotin-FITC uptake were studied using a thin film model originally developed to study the sorption and desorption of gases in polymers. This model assumes that the sorption process is irreversible and that diffusion into the interior of the film is due to transport through the matrix. The approximation to the diffusion equation for this system is:

$$\frac{D}{\varepsilon} \frac{\partial^2 C}{\partial x^2} + \frac{\varepsilon}{D} \frac{\partial C}{\partial t} = 0$$
where \( X_t \) is the total amount of biotin absorbed at time \( t \), \( X_s \) is the amount corresponding to biotin saturation, \( \ell \) is the nanowire radius, and \( D \) is the effective diffusion coefficient. The lines in Figure 3.13 are best fits of Equation 3.1 to the experimental data for \( \chi_{\text{biotin}} < 80\% \), and \( D \)’s are determined to be \( 5.4 \times 10^{-15} \, \text{cm}^2/\text{sec} \) and \( 1.2 \times 10^{-15} \, \text{cm}^2/\text{sec} \) for nanowires synthesized at constant \( E \) and \( E \) cycling, respectively. Nanowires synthesized at constant \( E \) have faster accessibility to the protein binding sites embedded in the Ppy matrix than those made by \( E \) cycling. This result is consistent with the cyclic voltammetry experiments in section 2.3.2 of chapter 2 (Figure 2.11), which showed evidence of slow mass transport of charge-compensating anions (which are small compared to biotin-FITC) in Ecy nanowires. The faster diffusion of both electrochemical species and biotin-conjugates in Eco nanowires seems likely due to their open internal structure (inset Figure 3.7d). Indeed, the differences in the internal structures of Eco and Ecy nanowires seem to play a significant role in the kinetic behavior of the Ppy nanowires.

### 3.4 Conclusions

The proteins avidin and streptavidin were successfully incorporated in Ppy nanowires by entrapment during polymerization. Assays on the number of binding sites in protein-modified nanowires demonstrate that nearly all proteins present in the polymer
matrix retained their ability to bind their natural substrate, biotin. The use of avidin and streptavidin affinity to biotin, or biotinilated fluorescence probes, validated this biological system as a great tool for the quantitative analysis and characterization of avidin/streptavidin-modified Ppy nanowires.

It was determined that the protein net charge had little effect on the amounts incorporated in the nanowires. Therefore, the doping mechanism of proteins into conducting polymers is not a major contributor in this system. Instead, an equilibrium concentration (given enough equilibration time) is maintained near the electrode surface and proteins are entrapped as the polymer matrix grows. Fluorescence images confirmed an even distribution of fluorescently labeled proteins throughout the polymer segment.

The kinetics of biotin binding to protein-modified nanowires showed slow diffusion of biotin species into the polymer nanowires. The slow diffusion resulted almost certainly from the extremely smooth and continuous surface of the polymer nanowires as seem by AFM, SEM, and TEM; this is unfavorable for the diffusion of large molecules into the polymer. In fact, voids in the polymer matrix were undetectable. Nevertheless, small molecules are able to diffuse into the Ppy nanowires, and this property can be applied to other nanostructured systems. Further use of the entrapment of biomolecules can be found in Chapter 4.
3.5 References


5 Cui, Y; Wei, Q.; Park, H.; Lieber, C.M. *Science* **2001**, 293, 1289.


Figure 3.1: Schematic of (a) tetramer crystal structure of avidin and biotin chemical formula, (b) proteins in polymer thin films, (c) proposed protein-modified polymer nanowires.
Figure 3.2: Schematic of the proposed entrapment mechanism of proteins into nanowires during polymerization at pH 9. The incorporation of species by the doping mechanism proposes that negatively charge molecules will be attracted to the positively charged active polymer surface.
Figure 3.3: Schematic diagram of the methods used to quantify the amount of protein binding sites in Ppy nanowires.
Figure 3.4: Diagram of the step by step procedure employed to measure biotin binding kinetics to avidin or streptavidin-modified Ppy nanowires.
Figure 3.5: Detection of the presence of avidin in polypyrrole nanowires by UV-VIS spectroscopy at 280 nm, (a) spectra of 4 µM avidin solution in PBS, pH 7.4, (b) spectra of Ppy nanowires, (c) spectra of avidin-modified Ppy nanowires. All nanowires were suspended in PBS, pH 7.4.
Figure 3.6: (a) Bright-field and (b) fluorescence images of Au/Ppy/Au nanowires containing avidin-RITC in the Ppy matrix, excited at 488 nm, recorded at λ > 512 nm.
Figure 3.7: (a) Differential interference contrast (DIC), (b) false color fluorescence, (c) overlapped DIC and fluorescence images, and (d) close view of the overlap image of Au/Ppy nanowires containing avidin-Texas Red in the Ppy matrix, excited at 543 nm. Inset: FE-SEM image of the internal structure of Eco nanowires.
Figure 3.8: Illustration of UV-VIS absorbance measurements for the determination of avidin and streptavidin in polypyrrole nanowires from absorption measurements of biotin-FITC conjugates by UV-VIS spectroscopy at 494 nm.
Figure 3.9: Binding sites in avidin/streptavidin-modified Ppy Eco nanowires as a function of pH. Total binding sites in streptavidin (♦) and avidin (▲) modified nanowires; available binding sites after 10 hours in streptavidin (□), and avidin (○) modified nanowires.
Figure 3.10: (a) Influence of polymerization potential on streptavidin incorporation in Ppy nanowires synthesized from 0.2 M pyrrole in PBS, at pH 5.5 (■), and pH 9 (●). (b) Streptavidin and avidin incorporated into nanowires synthesized at pH 9, as a function of constant oxidation potential.
Figure 3.11:  (a) Growth rate as a function of oxidation potential for Eco nanowires grown at pH 5.5 (●), pH 7.4 (□), and pH 9 (▲). (b) Binding sites in streptavidin-modified Ppy nanowires synthesized by a constant potential of 0.75 V, and by cycling the potential from -0.2 V to 0.9 V vs. SCE.  Inset: growth rate of Eco nanowires (●), and Ecy nanowires (◊).
Figure 3.12: Illustration of UV-VIS absorbance measurements of biotin-4-FITC remaining in supernatant solution for the study of binding kinetics of avidin or streptavidin modified nanowires.
Figure 3.13: Adsorption kinetics for biotin-FITC binding to Ppy nanowires. Streptavidin-Ppy nanowires (□), and unmodified nanowires (▲) synthesized by constant E. Streptavidin-Ppy nanowires (△), and unmodified nanowires (▲) synthesized by E cycling. Lines are best fits to eq 1.
Chapter 4

Polymer Nanowires with Catalytic Properties

4.1 Introduction

Nearly all applications of conducting polymers in catalytic systems involve the use of the polymer material primarily as an immobilizing medium or as an electron transfer conduit on an electrode surface, with the real catalytic function performed by the immobilized species, rather than the polymer. However, it was reported that conducting polymers have shown good electrocatalytic properties.\(^1\) Interestingly, research on metal plating applications led to the discovery that the undoped form of polyaniline is able to reduce noble metal ions that have low oxidation potential such as Ag\(^+\) and Pd\(^{2+;4+}\) spontaneously.\(^2\) Additionally, studies of electroplating rates on conducting polymer substrates showed significant enhancement compared to electroplating onto gold substrates.\(^3\) Nevertheless, the catalytic properties of conducting polymers are limited and, thus, they are not well known for their inherent catalytic activity and are considered to be catalytically inactive for the most part. In contrast to conjugated polymers, there also exists another class of “redox polymers”. Typically, a redox polymer consists of a system where a redox-active transition-metal-based pendant group is covalently bound to some sort of polymer backbone, which may or may not be electroactive. The goal of coating electrodes with electroactive polymers is the development of new materials with very active catalytic properties.

The immobilization of enzymes into polymeric materials has also emerged as an important method for the development of active catalytic materials with important
Current methods of enzyme immobilization include adsorption or covalent attachment to a support, microencapsulation, and entrapment within a membrane, film, or gel. Furthermore, there is an enormous interest in using nanoparticles for enzyme encapsulation, biosensors, and drug delivery. Enzyme-modified nanowires and nanotubes can utilize much of the already established self-assembly methods and interrogation platforms for anisotropic nanoparticles to develop novel nanoscale biosensor systems and biofuel cells. The immobilization of enzymes onto carbon nanotubes has been successfully accomplished by several groups for used in amperometric catalytic sensors of glucose and in the hydrolysis of penicillin. However, limitations in biocompatible fabrication schemes for nanowire systems have made enzyme encapsulation into nanowires a difficult task.

The focus of this chapter is the incorporation of the enzyme catalase into conducting polypyrrole (Ppy) nanowires to serve as catalyst centers, where the polymer itself is inert and serves only as a support for the catalytic entity. The insight gained from the work presented in the preceding chapter (the most favorable conditions for protein incorporation during polymer electrodeposition) allowed the successful encapsulation of active catalase into Ppy nanowires.

Enzymes are very large and complex organic molecules that are important because they speed up the rate of the reaction they catalyze that would otherwise be too slow to support life. Catalase is an enzyme present in the cells of plants, animals and aerobic (oxygen requiring) bacteria. It promotes the conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent of cells, to water and molecular oxygen.

$$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2,$$  \hspace{1cm} (4.1)
In the absence of catalase, this reaction occurs spontaneously, but very slowly. Catalase has one of the highest turnover rates for all enzymes: one molecule of catalase can convert 6 million molecules of hydrogen peroxide to water and oxygen each minute, in fact, catalase is one of the most potent catalysts known. Bovine liver catalase, the specific enzyme used in this work, has a molecular weight of 250,000 with four subunits of equal size. Its optimum pH is 7.0 with an isoelectric point is 5.4. Each molecule of catalase is a tetramer of four polypeptide chains. Each chain is composed of more than 500 amino acids. Located within this tetramer are four porphyrin heme groups that are responsible for catalase’s enzymatic activity. Encapsulated or immobilized catalase is used in the food industry whenever hydrogen peroxide needs to be destroyed, for example, in the manufacture of cheese. The mechanism of catalase catalysis is not yet precisely understood, but the following, which is similar to the mechanism of cytochrome c peroxidase, has been proposed. The catalytic process is thought to occur in two stages:

\[
\begin{align*}
H_2O_2 + Fe(III)-En & \rightarrow H_2O + O=Fe(IV)-En & (4.2) \\
H_2O_2 + O=Fe(IV)-En & \rightarrow H_2O + Fe(III)-En + O_2 & (4.3)
\end{align*}
\]

where Fe-En represents the iron center of the heme attached to the rest of the enzyme (En).

Catalase was incorporated into Ppy segments of segmented gold/Ppy nanowires by using the binding affinity between avidin-modified Ppy nanowires as developed in Chapter 3 and biotinylated catalase conjugates, and by the physical entrapment of the enzyme during polymer electrodeposition.

The catalytic activity and solution kinetics of gold/catalase-modified Ppy nanowires were compared to segmented Pt/Au nanowires developed by Paxton et al. In
their work, Paxton et al. observed the non-Brownian motion of Pt/Au nanowires in dilute solutions of hydrogen peroxide. A model was proposed for the motion of these nanowires based on an interfacial surface tension gradient caused by the decomposition reaction of hydrogen peroxide into water and molecular oxygen (Figure 4.1). In this model, the decomposition of hydrogen peroxide is considered to occur solely on the Pt surface. Paxton proposed that the observed movement of the Pt/Au nanowires with the Au segment moving forward resulted from the system’s attempt to minimize its interfacial energy by moving away from the high end of the oxygen concentration gradient. A subsequent model designated a more active role for the Au segment and suggested that the electrochemical decomposition of hydrogen peroxide occurred at both Pt and Au ends. In addition to nanowires containing enzyme catalyst, gold/Ppy nanowires loaded with platinum nanoparticles in the polymer segment were developed to elucidate the relationship between materials properties and motility.

The work presented in this chapter has been the result of a collaboration between the Ayusman Sen and the Thomas Mallouk research groups in the chemistry department at the Pennsylvania State University (PSU). In particular, Mr. Timothy Kline (Sen’s group) and Ms. Julia Bingham (Mallouk’s group), who worked in these studies, should also be regarded as co-authors of this work.
4.2 Experimental Methods

4.2.1 Materials

Anodic alumina membranes (Whatman Inc., NJ) containing cylindrical pores ca. 0.3 µm in diameter were employed as the templates for nanowire growth (Appendix A). A thin layer (150 nm) of sacrificial Ag was deposited on the branched side of the membranes by thermal evaporation to serve as the working electrode. 2,3-dihydrothieno[3,4-b]-1,4-dioxin (also known as 3,4-ethylene dioxothiophene, EDOT), pyrrole (98+%), catalase (20 mg/mL), ferrocene (98%), H₂SO₄ (99.999%), bovine serum albumin (BSA), urea (>98%), mercapto ethylamine (MESA), NaOH (97+%), and methanol (99.96%) were obtained from Sigma-Aldrich. Platinum powder (325 mesh, 99.9+%), and H₂PtCl₆ (99%) were obtained from Alfa Aesar. Silver (1025) and Au (Orotemp 24) electroplating solutions were obtained from Technic Inc., Cranston, RI. Avidin, NHS-fluorescein [5-(and 6)-carboxyfluorescein, succinimidyl ester], sulfo-NHS-LC-LC-biotin [sulfosuccinimidyl-6-(biotinamido)-6-hexanamido hexanoate], a microdialyzer system (5000 MW), and neutravidin-polystyrene beads were purchased from Pierce (Rockford, IL). Ultrapure water (18 MΩ-cm) was used for the preparation of all solutions and for rinsing. PBS solutions were prepared with 0.1 M NaCl (99.0%, Sigma), 0.003 M KCl (99.7%, J.T.Baker), and 0.002 M total phosphate concentration using potassium dihydrogen phosphate (99+%, Sigma) and disodium hydrogen phosphate (100%, J.T.Baker) to obtain pH values of 7.4. Phosphate buffer (PB) solutions were prepared with 0.1 M KBr (99.99%, Aldrich), 0.05 boric acid (>99.5%, Sigma), 0.05 M sodium dihydrogen phosphate (>99.0%, Sigma), and 0.1 M sodium acetate (>99.5%, Fluka), adjusted to pH 7 by addition of NaOH.
4.2.2 Characterization

Optical imaging was achieved with an Olympus BX60M reflected-light microscope equipped with an Olympus U-CMAN-2 camera (Olympus, Japan). TEM images were obtained with a JEOL 1200 EXII at a 80 kV accelerating voltage. Electrochemical synthesis, characterization and manipulation were achieved using the electrochemical system described in the next section. In addition, an oil immersion inverted microscope (Axiovert S100TV, Carl Zeiss) equipped with a mercury-xenon arc lamp (Opti Quip) and a halogen light source was used to acquire fluorescence images. Absorbance measurements were performed with a diode array spectrophotometer (HP 8452A).

4.2.3 Electrochemical System

Electrochemical synthesis and experiments were performed using a potentiostat/galvanostat (Bioanalytical Systems, model 100B or an EG&G Princeton Applied Research, model 363) in a one-compartment, three-electrode cell at room temperature (Appendix A). A platinum foil (2 cm$^2$) served as a counter electrode. A saturated calomel electrode (SCE), a silver/silver chloride (Ag/AgCl) or a silver wire served as the reference electrodes when specified. A silver wire was employed for the most part as pseudo-reference electrode during the synthesis of PEDOT nanowires and was calibrated using a ferrocenium/ferrocene (Fc$^+/Fc$) couple ($E^* = 0.400$ V vs. NHE)$^{21}$ as an internal standard to determine its potentials.$^{22}$
A thin layer (150 nm) of sacrificial Ag was deposited on the branched side of the anodic alumina membranes by thermal evaporation. A gold disk electrode (1.5 mm in diameter, BAS) was used as the working electrode for synthesis and characterization of conducting polymer thin-films.

4.2.4 Synthesis of Gold/polymer Nanowires

The synthesis and characterization of Ppy nanowires is described in detail in Chapter 2. Chapter 2 presented a comprehensive study of the optimal experimental conditions for the synthesis of Ppy nanowire segments from buffer solutions, providing a biocompatible environment suitable for incorporation of biomaterials into these systems. Ppy nanowires were synthesized by a template replication method\textsuperscript{23} as depicted in Appendix A. The templates used in this work were alumina membranes characterized by having a branched side and an unbranched side that result from the anodization process employed to produce cylindrical pores.\textsuperscript{24} Approximately 3-4 µm of additional sacrificial silver was electrodeposited into the alumina membrane to fill the branched section of the template and prevent branched nanowire structures.

The fabrication of segmented gold/conducting polymer nanowires inside the templates is depicted in Figure 4.2. Electro-deposition of Au was carried out at a constant potential of −0.9 V vs. SCE using a commercial Au plating solution containing approximately 6.9% potassium aurocyanide (KAu(CN)\textsubscript{2}). Au-containing membranes were soaked in pyrrole solutions for 10 minutes prior to polymerization.

Ppy nanowires were grown from 0.2 M pyrrole solutions in 150 mM PBS at pH 7.4 at a constant potential of 0.85 V to obtain a segment length of ~1.5 µm; for a total
nanowire length of 3 µm. The alumina membranes were subsequently treated with 25% (v/v) HNO$_3$ and 0.5 M sodium hydroxide to obtain freestanding nanowires. Nanowires were rinsed five times with water and re-suspended in water.

4.2.5 Catalase-modified Gold/polypyrrole Nanowires

4.2.5.1 Catalase Immobilization by Avidin-biotin Binding

The avidin-biotin binding affinity was used to immobilize catalase to Ppy nanowires containing avidin proteins embedded in the polymer matrix (Figure 4.2, scheme A). The fabrication of avidin-modified Ppy nanowires was studied in Chapter 3 and was employed in this work to bind biotinylated catalase to avidin proteins in the polymer segment of Au/Ppy nanowires.

Biotin moieties and fluorophores were attached to catalase by standard labeling techniques used in molecular biology (see Figure 4.3). A 3 mL solution containing 5 mM NHS-fluorescein, 5 mM sulfo-NHS-LC-LC-biotin, and 3.6 mg of catalase were incubated in an ice bath for 2 hours. Unreacted NHS-fluorescein and sulfo-NHS-LC-LC-biotin were removed by dialysis using a membrane with molecular weight cut off of 5000 Daltons. Aliquots of 500 µL were stored at -20 °C until use. A biotin-catalase conjugate was also prepared using the same procedure. The catalase-biotin/fluorescein conjugate was analyzed by western blot with the assistance of Ms. Sonalee Athavankar (Chemistry, PSU) to determine the quality of the protein-biotin conjugate. Western blot allowed the determination of the molecular weight of the catalase protein present in the system and its biotin/fluorescein conjugates and their relative amounts. A detailed explanation of the western blot procedure can be found in Appendix C.
Catalase-biotin/fluorescein conjugates were incubated at 37 °C in separate assays with Au/avidin-modified Ppy nanowires (see section 3.2.5 for procedure on immobilization of avidin in Ppy nanowires), protein-free Ppy nanowires, and neutravidin-modified polystyrene beads. Nanowires were treated with BSA to reduce the nonspecific binding of the proteins to the surface of gold segments. Nanowires and beads were rinsed exhaustively to remove any unbound biotin/fluorescein-catalase conjugate.

4.2.5.2 Catalase Immobilization by the Entrapment Method

Segmented Au/catalase-modified Ppy nanowires were prepared using a physical entrapment method to immobilize proteins into the polymer segment (Figure 4.2, scheme B). In general, when the appropriate potential for polymerization is applied to the working electrode immersed in a solution containing both protein and monomer molecules, the proteins in the vicinity of the electrode surface are incorporated into the growing polymer. A more detailed discussion of the entrapment of proteins into Ppy is found in Chapter 3.

Catalase-modified Ppy wires were grown from solutions containing 0.2 M pyrrole and 5 mg/mL of catalase in PBS (pH 5.5, 7.4, and 9) at constant potential of 0.85 V. The pyrrole monomer was distilled and the solutions were freshly prepared before use. Nanowires were rinsed with PBS solution and resuspended in PBS until use.

4.2.6 Synthesis of Polypyrrole Films and Catalase-modified Polypyrrole Films

A 1.5 mm in diameter gold disk electrode was used to study Ppy films. The electrode was cleaned with piranha solution (3:1 mixture of sulfuric acid and 30%
hydrogen peroxide) followed by electrochemically cleaning in a 0.5 M H$_2$SO$_4$ solution by
cycling the potential from -0.2 V to 1.7 V vs. SCE prior to every experiment. The disk
electrode was tested periodically in a ferrocene solution by cycling the potential between
0 and 0.4 V vs. SCE at 100 mV/s to examine the quality and cleanliness of the gold
surface. The pre-cleaned electrode was rinsed and stored in monomer solution prior to
polymerization. Conducting Ppy films were grown onto the gold disk electrode by
anodic oxidation from 0.2 M pyrrole solutions in 150 mM PBS or phosphate buffer (PB),
pH 7.4 at 0.85 V vs. SCE for a total of \(~3.4 \times 10^{-2}\) Coulombs of charge passed through
the electrode.

Catalase-modified Ppy films were grown on a pre-cleaned gold disk electrode
from solutions containing 0.2 M pyrrole and 5 mg/mL of catalase in PBS (pH 7.4) at
constant E of 0.85 V vs. SCE for a total of \(~3.4 \times 10^{-2}\) Coulombs of charged. Films were
immersed in PBS solution when not in use; nevertheless, films were freshly prepared
before use.

4.2.7 Fabrication of Platinum Filled Gold/polymer Nanowires

4.2.7.1 Incorporation of Pt Nanoparticles by Filtration of Nano-powder

Gold segments were grown prior to polymer deposition. Au was electrodeposited
at a constant potential of \(~0.9\) V vs. SCE using a commercial Au plating solution
containing approximately 6.9% potassium aurocyanide (KAu(CN)$_2$) for a total length of 1
µm. The Au-containing membrane was rinsed with acetone, dried with nitrogen, and
sonicated for 20 minutes in a solution containing 0.05 M 3,4-ethylene dioxythiophene
(EDOT) monomer, 0.1 M tetrabutyl ammonium perchlorate, and 10 mg/mL of platinum
powder (see inset in Figure 4.17 for TEM image) in methylene chloride. Sonication of the membrane in the presence of Pt particles promoted the introduction of nanoscopic particles into the pores of the alumina template. Poly(3,4-ethylene dioxythiophene) (PEDOT) segments were grown by scanning the potential between 0.3 V to 2.2 V vs. E'\((\text{Fe}^+/\text{Fe})\) (a silver wire was used as a pseudo-electrode) at 20 mV/s for 6 cycles to produced segments of approximately 2 µm long. The alumina membranes were subsequently treated with 25% (v/v) \(\text{HNO}_3\) to remove the sacrificial silver, and 0.5 M sodium hydroxide to dissolve the alumina membrane and obtain free-standing nanowires. Nanowires were rinsed approximately five times with water and then re-suspended in water for subsequent experiments.

### 4.2.7.2 Incorporation of Pt Nanoparticles by Direct Reduction of PtCl\(_2\)\(^{2-}\)

Au/Ppy nanowires were fabricated by the electrodeposition of gold, followed by the electropolymerization of Ppy in alumina templates. Ppy segments were polymerized from an aqueous solution containing 0.3 M pyrrole and 1 M \(\text{H}_2\text{SO}_4\) at a current density of 1.9 mA/cm\(^2\). Pt nanoparticles were incorporated into Ppy by cycling the membrane potential between -0.2 and 0.8 V vs. SCE at a scan rate of 100 mV/s in an aqueous solution of 10 mM \(\text{H}_2\text{PtCl}_6\) and 1 M \(\text{H}_2\text{SO}_4\).\(^{27}\)

### 4.2.8 Oxygen Evolution Studies

The rate of oxygen evolution from the decomposition of \(\text{H}_2\text{O}_2\) (equation 4.1) was used to determine the catalytic activity of the biocatalyst (catalase) and the inorganic catalyst (platinum) used to modified gold/polymer nanowires. A 500 µL water
suspension of $\sim 10^6$ gold/polymer or catalyst-modified gold/polymer nanowires/mL was placed in a septum-capped tube and the system was purged with argon for 20 minutes. Another 500 µL of an aqueous solution of 0.5% hydrogen peroxide was added to the nanowire suspension. Gas samples were removed from the test tube periodically as the reaction proceeded and analyzed for oxygen content by gas chromatography (Buck Scientific 910) using a thermal conductivity detector at room temperature and a molecular sieve 15 m column (Supelco). The amount of oxygen emerging from leaks in the system was determined by monitoring the nitrogen content in the system and using the physical ratio of oxygen to nitrogen content in the atmosphere; and was subtracted from the final calculation.

4.2.9 Autonomous Movement Studies

Particle tracking is the method employed to study the motion of particles in solution. In general, a 25 µL suspension containing $\sim 10^6$ nanowires/mL and 3-10 w% hydrogen peroxide was placed in a sealed well on a clean glass slide and covered with a glass cover slip. The movement of suspended nanowires was captured by video and analyzed to determine the properties of their motion. Nanowires were tracked by center-to-center displacement along the longest axis of the nanowires as a function of time. Velocities, directionalities, and diffusion coefficients of individual nanowires can be obtain by this tracking method.
4.3 Results and Discussion

4.3.1 Gold/Ppy Nanowires

Figure 4.4 shows an optical micrograph and TEM image of the ideal Au/Ppy nanowires with both gold and polymer segments of 1 µm in length. The purpose of this segmented structure was to reproduce the Pt/Au model developed by Paxton et al. by replacing the platinum segment with a polymer segment with superior catalytic strength. The protein catalase and platinum nanoparticles were chosen to impart the Ppy matrix superior catalytic activity compared to solid Pt, and ultimately fabricate faster catalytic nanomotors.

4.3.2 Properties of Catalase-modified Gold/polypyrrole Nanowires

Two methods were used to immobilize catalase into Ppy nanowires: the binding affinity of the avidin-biotin complex, and the physical entrapment during the polymer synthesis (Figure 4.2).

4.3.2.1 The Avidin-biotin Binding Affinity

Figure 4.3 shows a schematic diagram of the biochemical approach taken in this chapter for the immobilization of catalase on Ppy nanowires using the avidin/ biotin binding system. The biotin/fluorescein-catalase conjugate was prepared in house and was reacted with neutravidin-modified polystyrene beads and shown in Figure 4.5. The fluorescence image reveals that fluorescein was immobilized on the beads and such immobilization resulted from the high binding affinity of the molecule biotin to the neutravidin on the polystyrene surface.
Au/avidin-modified Ppy nanowires were reacted with the biotin/fluorescein-catalase conjugate to immobilize catalase onto the Ppy segment, and thus impart catalytic properties to the polymer. Figure 4.6 shows a typical optical and fluorescence image of nanowires showing non-specific binding of the catalase conjugate to the gold surface. Several approaches were taken to minimize the binding of catalase to the gold surface of nanowires, including incubation of the nanowires with a blocking agent such as BSA prior to reacting with the catalase conjugate. BSA is commonly used to passivate metallic surfaces by spontaneously absorbing onto the surface forming a hydrophobic monolayer of protein material that may prevent any reactivity of the surface. Nevertheless, results like those in Figure 4.6 were typical of even nanowires pre-treated with BSA.

The catalytic activity of free catalase, biotin-catalase conjugate and reacted nanowires was studied by quantifying the amount of oxygen evolved from the catalytic decomposition of hydrogen peroxide to water and oxygen and is shown in Figure 4.7. The catalytic activity of catalase is maintained throughout the conjugation process. Further studies on the autonomous movement of these nanowires showed only Brownian motion; hence the absence of any directional propulsion force. This result was not surprising since Figure 4.6 revealed that both gold and polymer segments were covered with catalase conjugate and lacked asymmetry in the placement of the catalyst.

The complexity of the approach in Figure 4.3 contributed to the failure to achieve the proposed system and much work was required to identify the limiting step in this extensive fabrication process. One problem was discovered during the western blot analysis of biotin/fluorescein-catalase conjugates synthesized in house, as shown in
Figure 4.8. Although western blot showed that the synthetic approach used for the labeling of catalase with biotin and fluorescein molecules was successful (shown by the appearance of blue bands, indicating the presence of fluorescein in lanes 1-4 and biotin in lanes 5-9), much of the product of the conjugation was low molecular weight protein fragments represented by the smearing of the bands in Figure 4.8.

4.3.2.2 The Entrapment Method

Immobilization of catalase onto Ppy segments was successfully accomplished by the physical entrapment method of the protein during Ppy growth. Once again, this method proved to be successful for incorporating another protein into Ppy, similar to the work presented in Chapter 3. Catalase-modified Ppy segments were grown from solutions containing 0.2 M pyrrole and 5-10 mg/mL of catalase in PBS (pH 7.4) at constant potential of 0.85 V to obtain catalase-modified Ppy segments of 2µm in length and ~300 nm in diameter.

Enzyme Activity: The rate of oxygen production by the catalytic activity of catalase embedded in Ppy segments is shown in Figure 4.9. The rate of oxygen generation of Au/catalase-modified Ppy nanowires was experimentally determined to be $8.8 \times 10^{-16}$ mol O$_2$/s per nanowire. Although the amount of catalase per nanowire was not quantified, catalase has similar characteristics (size and isoelectric point) as the streptavidin proteins immobilized in Ppy nanowires and studied in Chapter 3. By assuming that the amount of streptavidin entrapped in Ppy nanowires (900 biotin binding sites or 225-900 protein molecules per 1 µm long Ppy segment of 300 nm in diameter at pH 7.4, from Figure 3.9) is similar to the amount of catalase in Ppy nanowires
synthesized with the same experimental conditions, and that one molecule of catalase can convert 6 million molecules of hydrogen peroxide to water and oxygen each minute, it is estimated that Au/catalase-modified Ppy with polymer segments of 2 µm would exhibit a rate of oxygen evolution between $4 \times 10^{-17}$ and $1.5 \times 10^{-16}$ mol O$_2$/s per nanowire. This range is slightly lower than the experimental value of $8.8 \times 10^{-16}$. Figure 4.8 showed that catalase used in this study was composed of tetramers and fragments of protein, all containing the porphyrin heme group responsible for catalase’s enzymatic activity. It is likely that fragments of catalase were incorporated into Ppy nanowires at higher loadings than a sample composed of pure tetramer due to smaller steric hindrance and higher accumulation in the pores. This could explain the higher experimental value for the rate of oxygen evolution in Au/catalase-modified Ppy nanowires.

Figure 4.9 also shows the effect of urea, a commonly used enzyme inhibitor, on the catalytic activity of Au/catalase-modified Ppy nanowires. Denaturation of proteins is the result of unfolding of the monomer units and is commonly achieved by disrupting the intermolecular forces (mainly hydrogen bonding) that gives a protein its ternary and quaternary structure. In this work, Au/catalase-modified Ppy nanowires were exposed to 6 M urea for up to 24 hours. It was determined that catalase embedded in Ppy nanowires lost 10% of its activity after exposure to urea for 1 hour and <30% activity after exposure for 24 hours. Although catalase is known to be robust compared to other enzymes, these results are remarkable when compared to other work on the catalytic activity of free and immobilize catalase. For example, studies on the effects of various inhibitors, including urea, on the activity of catalase from chicken erythrocytes determine that the free enzyme lost 88 % of its activity after incubation in 4 M urea after 1 hour. In another report,
Betancor and coworkers\textsuperscript{29} studied the immobilization of catalase and concluded that the stabilization of the quaternary structure of catalase is critical in order to prevent inactivation. In their work, catalase was immobilized by multi-step covalent bonding on different supports and chemical cross-linking. The optimal protein stabilization achieved by their work retained 60\% activity of the immobilized catalase. The entrapment method employed to introduce catalase to Ppy nanowires not only imparted the nanowires with catalytic properties, but also prevented the enzyme inactivation, perhaps by locking the enzyme’s quaternary structure into place during an in situ cross-linking mechanism in the growing Ppy matrix and/or by slowing the diffusion of the enzyme inhibitor into the nanowire.

\textbf{Autonomous Movement:} The movement of Au/Pt nanowires in aqueous hydrogen peroxide is characterized by non-Brownian motion; with motion in the direction of their long axis and a rate of oxygen evolution of $9.7 \times 10^{-16} \text{ mol O}_2/\text{s per nanowire}$ as studied by Paxton et al. In this study, and interfacial surface tension gradient model was proposed as the mechanism for the motion of Pt/Au nanowires in dilute hydrogen peroxide solutions, and correctly predicts the observed nanowire velocity.

Au/catalase-modified Ppy nanowires presented an opportunity to test the interfacial gradient model. Studies on the catalytic activity these nanowires (Figure 4.9) resulted in a rate of oxygen evolution of $8.8 \times 10^{-16} \text{ mol O}_2/\text{s per nanowire}$, on the same order as Paxton’s Pt/Au nanowires. Nevertheless, Au/catalase-modified Ppy nanowires did not exhibit autonomous movement. Although these nanowire systems are chemically different in nature, they both decompose hydrogen peroxide at one end of the nanowire at
a similar rate. This result suggests that the motion of Pt/Au nanowires may entail more than an interfacial gradient mechanism, and that the chemical and electronic properties of the materials involved in the catalytic reaction may play a very important role.

Scientists, including Paxton, in the chemistry department at PSU are currently developing an alternative model to explain the propulsion of anisotropic particles by the catalytic decomposition of hydrogen peroxide that accounts for the electrochemical properties of the materials in the system. The new model based on electrokinetic pumping of fluid generated by the catalytic activity of the nanowire, incorporates the significance of the electrical contact between each end of the nanowires and proposes a nanoscale fuel-cell system that transfers electrochemical power into mechanical propulsion. This model assumes that only about 1% of the oxygen generated by the catalytic reaction is responsible for the movement of the nanowires. As discussed before, the rate of oxygen generated by Pt/Au nanowires with autonomous movement was determined to be $9.7 \times 10^{-16}$ mol O$_2$/s per nanowire. If an electrochemical mechanism is responsible for the motion of these nanowires, then a current of $10^{-12}$ Amps is estimated to be the lower limit of electrical current in a nanowire with a cross section of 300 nm in order to exhibit non-Brownian motion. In fact, the electrical characteristics of the Ppy matrix encapsulating the biocatalyst (catalase) in Au/catalase-modified Ppy nanowires were characterized in Chapter 2 (Figure 2.20) and showed that these nanowires can pass maximum currents on the order of $10^{-12}$ Amps. The Ppy matrix in these nanowires had insulating characteristics that could explain the absence of propulsion in Au/catalase-modified Ppy nanowires.
4.3.3 Electrochemical Properties of Polypyrrole Films and Catalase-modified Polypyrrole Films

The previous section concluded that the interfacial tension mechanism proposed by Paxton et al. to explain the non-Brownian movement of Pt/Au nanowires cannot explain the lack of mobility of Au/catalase-modified Ppy nanowires, despite the fact that both systems exhibited similar rates of oxygen production. A new electrochemical mechanism, however, may give insight into the properties necessary to fabricate biocatalytic nanomotors.

Ppy films and catalase-modified Ppy films were synthesized using identical experimental parameters as their nanowire counterpart to elucidate the electrochemical properties of these materials. All films discussed in this section were polymerized to for a total of ~3.4 x 10^{-2} coulombs of charge passed through the electrode to achieve uniform film thickness. Figure 4.10a shows the cyclic voltammogram (CV) of a Ppy film polymerized onto a gold disk electrode compared to that of the bare gold surface in a ferrocene solution. The redox process of the ferrocenium/ferrocene couple can be observed for the Ppy film electrode, despite the typical capacitive superimposed wave resulting from the polymer’s microporosity. Thicker Ppy films did not exhibit redox peaks. This suggest that the thickness of the Ppy film used in this work was appropriate for analysis of electroactivity. Figure 4.10b shows a typical CV for the electrochemical behavior of Ppy in PBS solution.

The direct electrochemistry of catalase entrapped in Ppy films was studied by the reduction reaction involved in the redox couple of the heme porphyrin in catalase, the Fe^{III}/Fe^{II} couple. Several research groups have studied the electron transfer between
catalase and several electrode surfaces including catalase embedded in liquid crystals, single-wall carbon nanotubes (SWCNT) and chitosan films, and catalase covalently bonded to glassy carbon powder. In one example, the electrochemistry of catalase immobilized on gold/SWCNT electrodes showed well-defined redox peaks with a reduction peak at ~0.4 V vs. SCE and a peak separation of 32 mV for the oxidation and reduction peaks. They attributed this redox wave to the Fe^{III}/Fe^{II} redox center in catalase.

In order to investigate accurately the direct electrochemistry of catalase in Ppy films, Ppy was carefully studied in the potential range of 0 to -0.8 V vs Ag/AgCl to account for any artifacts that may arise from the polymer’s electrochemical behavior. Ppy films were synthesized from PBS solutions to reproduce the synthetic conditions of catalase-modified Ppy nanowires, and from phosphate buffer (PB) solutions to characterize and compare the electron transfer properties of the Ppy matrix by using similar experimental condition as reported in the literature.

Ppy films synthesized from PBS and PB solutions were scanned in the corresponding buffer immediately after polymerization and after storing the films for 24 hours in buffer solution (Figure 4.11). Storing the polymer films in buffer solution resulted in the appearance of redox peaks that immediately presented a problem to the study of catalase in these films. These peaks were noticeable after soaking the electrode for only 1 hour and became more defined after one day. Figure 4.12b compares Ppy films synthesized from both PBS and PB solutions. Both materials exhibited the appearance of redox peaks after 24 hours with a potential separation of ~150 and ~50 mV for the anodic and cathodic peaks, respectively. Previous work on catalase embedded onto chitosan-modified pyrolytic graphite electrodes states that chitosan provided a
favorable microenvironment for catalase to transfer electrons to the underlying pyrolytic graphite electrode, however, the study does not report the possible aging and buffer effects on the electrochemical properties of the chitosan films as observed in the Ppy films studied in this chapter. Their study on catalase-modified chitosan electrodes revealed that the peak intensities for the apparent redox wave from the Fe$^{III}$/Fe$^{II}$ redox center in catalase increased with time for up to 30 days of immersion in phosphate buffer. This increase was attributed to an increase in the amount of catalase embedded in the chitosan by diffusion of free enzyme from solution into the matrix. Nevertheless, Figure 4.11 suggests that aging of the electroactive surfaces may also give rise new peaks that can be mistaken for the redox reaction of the catalase Fe cofactor.

Ppy films and catalase-modified Ppy films were synthesized from both PSB and PB solutions and their electrochemical behavior was recorded immediately after polymerization. Figure 4.13a shows the CV of Ppy and catalase-modified Ppy films synthesized from PBS solution and scanned at 20 mV/s in PBS. An anodic peak is evident at -0.63 V vs. Ag/AgCl for the catalase-modified Ppy film. A comparison of the voltammogram of fresh catalase-modified Ppy and aged Ppy films can be seen in Figure 4.13b. Both samples exhibited anodic peaks with a separation of ~80 mV. Figure 4.14b shows the CV of fresh catalase-modified Ppy and aged Ppy films synthesized from PB solutions. Here, the redox peaks of the new catalase-modified Ppy film were overshadowed by the redox peaks of aged Ppy in PB buffer. The direct electrochemistry of entrapped catalase can not be studied by using PB solutions for the Ppy system. In addition to Figure 4.13a, Figure 4.15 shows the electrochemical behavior of fresh catalase-modified Ppy and fresh Ppy films synthesized from PBS solution and scanned at
5 and 200 mV/s in PBS. All three voltammograms of three different samples show distinct anodic peaks, evidence of electroactivity between catalase and Ppy.

Ppy nanowires synthesized in this chapter were exposed to sodium hydroxide to remove the alumina membrane and release the nanowires into solution. Therefore, Ppy films were also subjected to 0.5 M NaOH for 1 hour and an example of their electrochemical properties is shown in Figure 4.16. It is clear that Ppy loses its electroactivity upon exposure to NaOH. Immersion of the film back into PBS solution increases electroactivity, but the film never returns to its original state. This implies that catalase and Ppy nanowires may not be in electrochemical communication in the nanowires. If electrochemical electron transfer is necessary for the propulsion mechanism in the autonomous motion of Pt/Au nanowires discussed in the previous section, it may explain why Au/catalase-modified Ppy nanowires with similar catalytic power do not exhibit autonomous movement.

**4.3.4 Properties of Platinum Filled Gold/polymer Nanowires**

Au/polymer nanowires with platinum nanoparticles embedded in the polymer matrix were explored as an alternative system to catalase-modified Ppy nanowires. The inorganic nature of Pt allowed the use of severe chemistries that are not suitable for an enzyme. In the last decade, some research groups studied the incorporation of transition metals (e.g., Pt, Pd, Ru, Sn) microparticles in conducting polymer-coated electrodes which exhibited enhanced electrocatalytic activities compared to the bulk-form metal electrodes.27,30,31 Similarly, Pt nanoparticles are expected to exhibit higher catalytic activity than Pt segments due to a higher surface area.
Poly(3,4-ethylene dioxythiophene) (PEDOT) was used in this work to fabricate Au/Pt-filled PEDOT nanowires. PEDOT was selected for its superior electronic properties and chemical stability and it was studied in Chapter 5 as a model system for nanowire gas sensors. Electrical characterization on these polymer nanowires revealed that PEDOT nanowires are highly conducting even after exposure to NaOH.

Two methods were employed to incorporate Pt nanoparticles into the polymer segment: filtration of Pt nanoparticles into the pores of the alumina membrane prior to polymerization, and doping and electrochemical reduction of PtCl$_6^{2-}$ ions in a preexisting polymer segment. Figure 4.17 shows a TEM micrograph of a Au/Pt-modified PEDOT nanowire. The Pt loadings for Au/PEDOT nanowires varied greatly, from nanowires without particles to nanowires with ~60% loading that were mechanically unstable. The variability in the Pt loading in these nanowires presented a problem to study this system further. Samples were found to contain colloidal Pt particles that interfered with studies on the catalytic activity and motility. An alternative approach for the fabrication of Au/Pt-modified Ppy nanowires was employed. In the new method, PtCl$_6^{2-}$ ions were introduced and reduced into the polymer segment of Au/Ppy nanowires. Examples of the resulting structures are shown in Figures 4.18 and 4.19. A comparison of Figure 4.17 and 4.19 revealed that the direct reduction of PtCl$_6^{2-}$ ions into the polymer segment yielded nanowires with consistent Pt loading. In principle, the amount of Pt nanoparticles can be increased by further reduction of PtCl$_6^{2-}$.

Figure 4.20 shows the rate of oxygen evolved by the catalytic activity of Pt nanoparticles embedded in the Ppy segment of Au/Ppy nanowires and shown in Figure 4.19. A rate of $1.2 \times 10^{-15}$ mol O$_2$/s per nanowire was calculated for these nanowires, just
slightly higher than the rate of Pt/Au nanowires (1.0 x 10^{-15} mol O_2/s from data shown). It must be noted that the Au/Pt-Ppy nanowires studied have a Ppy segment shorter than 1 µm and that Ppy segments of equal size to Pt in Pt/Au nanowires should result in higher rates of oxygen evolution. The motion of Au/Pt-Ppy nanowires was studied in the presence of 5% hydrogen peroxide. The results were inconclusive: a few nanowires exhibited a circular movement; however, the majority of the nanowires displayed Brownian motion. There is no data available on the electrical properties of the Ppy surrounding the Pt particles; nevertheless, it is likely that Ppy is not electroactive.

### 4.4 Conclusion

Segmented Au/Ppy nanowires containing the enzyme catalase and Pt nanoparticles were successfully fabricated. When placed in dilute hydrogen peroxide solutions, Au/catalase-modified Ppy nanowires, Au/Pt-Ppy nanowires, and Pt/Au nanowires exhibited rates of oxygen evolution on the same order of magnitude. Nevertheless, only Pt/Au nanowires exhibited non-Brownian motion. The results reported in this chapter are not consistent with the proposed interfacial gradient model. However, they neither prove nor disprove the new electro-kinetic model that requires electroactivity of the entire nanowire structure. Other nanowires systems continue to be developed in order to elucidate the mechanism of the autonomous movement of nanowires with catalytic sites. Nanowires containing highly conducting polymer segments loaded with Pt nanoparticles are being investigated that may give some insight on the feasibility of the new proposed electro-kinetic model.
4.5 References


Figure 4.1: Schematic diagram of (a) the interfacial tension model for Pt/Au propulsion mechanism, and (b) trajectory plots of three 2 µm long Pt/Au nanowires in 2.5% aqueous hydrogen peroxide. Reproduced with permission of Paxton et al.¹⁹
3 µm of silver are electroplated at 0.5 mA/cm².

1 µm of gold is electroplated at 0.5 mA/cm².

Polypyrrole is electropolymerized at 0.85 V vs. SCE in the presence of avidin proteins.

Silver is dissolved in 25% v/v HNO₃. The alumina template is dissolved in 0.5 M NaOH.

Au/Ppy nanowires are reacted with biotin-catalase or biotin/fluorescein-catalase conjugates.

3 µm of silver are electroplated at 0.5 mA/cm².

1 µm of gold is electroplated at 0.5 mA/cm².

Polypyrrole is electropolymerized at 0.85 V vs. SCE in the presence of catalase proteins.

Silver is dissolved in 25% v/v HNO₃. The alumina template is dissolved in 0.5 M NaOH.

Figure 4.2: Schematic diagram of the synthesis of gold/polypyrrole nanowires and gold/catalase-modified nanowires by replication of alumina templates.
Figure 4.3: Schematic diagram of the biochemical approach for the immobilization of catalase on Ppy nanowires using the avidin/biotin binding system.
Figure 4.4: (a) Optical image, and (b) TEM image of Au/Ppy nanowires.
Figure 4.5: Optical micrographs of neutravidin-modified polystyrene beads reacted with the biotin/fluorescein-catalase; (a) differential interference contrast (DIC), (b) fluorescence image.
Figure 4.6: (a) Optical and fluorescence images of Au/avidin-modified nanowires reacted with the biotin/fluorescein-catalase. (b) Fluorescence of the entire nanowire revealed non-specific binding of the catalase conjugate to the gold surface.
Figure 4.7: Catalytic activity of catalase toward the decomposition of hydrogen peroxide to water and oxygen, analyzed by the amount of oxygen evolved in the system in free catalase (green circles), biotinylated catalase (blue squares), neutravidin-modified polystyrene beads reacted with biotinylated catalase (red triangles), avidin-modified nanowires reacted with biotinylated catalase (open squares), and catalase content on the supernatant solution from last rinsed after incubation of catalase complex with avidin-modified Ppy nanowires (yellow diamonds).
Figure 4.8: Picture of the western blot analysis of biotin/fluorescein-catalase conjugate
Figure 4.9: Rate of oxygen production by the catalytic activity of catalase embedded in Ppy segments of Au/Ppy nanowire sample as released from the template (OPEN squares), after exposure to 6 M urea for 1 hour (light blue squares), and after exposure to 6 M urea for 24 hours (deep blue squares). The catalytic activity of Pt/Au nanowires (red diamonds) is shown for comparison.
Figure 4.10: (a) CV of a gold disk electrode cycled in ferrocene solution between 0-400 mV vs. Ag/AgCl at 100 mV/s; bare gold surface (red), and Ppy film polymerized on a gold disk electrode surface (blue). (b) CV of a Ppy film polymerized on a gold disk electrode surface, cycled in PBS solution at 50 mV/s.
Figure 4.11: (a) Ppy films synthesized from PBS solution and scanned immediately after polymerization (red-dotted line) and after soaking in PBS for 24 hours (red-solid line). (b) Ppy films synthesized from PB solution and scanned immediately after polymerization (blue-dotted line) and after soaking in PBS for 24 hours (blue-solid line). All scans were performed at 20 mV/s.
Figure 4.12: Ppy films polymerized from PBS (red) and PB (blue), and scanned in the corresponding buffer solution at 20 mV/s. (a) Scanned immediately after polymerization, (b) scanned after storing in corresponding buffer solution for 24 hours. (c) (a) and (b).
Figure 4.13: (a) Ppy film (red-dotted line) and catalase-modified Ppy film (green-solid line) scanned immediately after polymerization. (b) Catalase-modified Ppy film scanned immediately after polymerization (green-solid line) compared to a Ppy film scanned after soaking in buffer for 24 hours. (c) (a) and (b). All scan were performed in PBS solutions at 20 mV/s.
Figure 4.14: (a) Ppy film scanned immediately after polymerization (blue-dotted line); Ppy film after soaking in buffer for 24 hours (blue-solid line); catalase-modified Ppy film scanned immediately after polymerization (red-solid line); and catalase-modified Ppy film after soaking in buffer for 72 hours (red-dotted line). (b) Ppy film scanned after soaking in buffer for 24 hours (blue-solid line); and catalase-modified Ppy film scanned immediately after polymerization. All scans were performed in PB solutions at 20 mV/s.
Figure 4.15: Ppy film scanned immediately after polymerization (blue) and catalase-modified Ppy film also scanned immediately after polymerization (red); (a) scan rate was 5 mV/s, (b) scan rate was 200 mV/s. All films were polymerized from PBS solutions.
Figure 4.16: Catalase-modified Ppy film, scanned immediately after polymerization (blue), after exposure to 0.5 M NaOH for 1 hour (red), followed by soaking in PBS solution for 1 hour (green). The scanning rate was 20 mV/s.
Figure 4.17: TEM image of an Au/Pt-filled PEDOT nanowire fabricated by filtration of Pt nanopowder into the alumina template and subsequent polymerization of PEDOT to encapsulate the catalyst. Inset: TEM image of Pt nanoparticles used in this system.
Figure 4.18: Optical micrograph of a Ppy film (a) before, and (b) after doping and direct reduction of \( \text{PtCl}_2^{2-} \).
Figure 4.19: TEM image of (a) a single Au/Ppy nanowire, and (b) multiple nanowires containing Pt nanoparticles at the polymer end by doping and direct reduction of PtCl$_2^{-}$. 
Figure 4.20: Rate of oxygen evolution by the catalytic activity of Pt nanoparticles (red squares) electrochemically grown in Ppy segments of Au/Ppy nanowires as seen in Figure 4.19. Oxygen evolution rates for Au/catalase-modified Ppy nanowires (open squares), and Pt/Au nanowires (green triangles) is shown for comparison.
Chapter 5

Nanowires Containing Electronic Polymer Junctions

5.1 Introduction

The earliest reported application of conducting polymers as sensors was the use of undoped polyacetylene to detect and to measure levels of iodine, bromine, and AsF$_5$ vapors doped into the polymer material inside Schlenk tubes.\textsuperscript{1} This simple sensor system inspired the early exploration of conducting polymers toward sensor applications,\textsuperscript{2} revealing the potential of these materials. Today, conducting polymers have found applications in molecular and opto-electronics, micro-actuators, and chemical and biological sensors,\textsuperscript{3} and they continue to be the main focus of numerous scientific investigations. Table 5.1 shows examples of conducting polymers that have found their place in the semiconductor and electronics market.

Conducting polymers possess many of the characteristics desired for sensor arrays. They display rapid, reversible changes in conductivity at room temperature combined with specificities that can be readily programmed by chemical modifications.\textsuperscript{4} Their conductivity can be tuned by chemical manipulation of the polymer chain, by the nature of the dopant, by the degree of doping, and by blending or copolymerizing with other materials. As a sensor component, conducting polymers may play a highly varied role. They may be active and serve as a catalytic layer, a redox mediator, an on/off switch, a resistor with a resistance value that is modulated by a targeted chemical reaction, or may contain the molecular recognition for a specific analyte. They may also be “inactive” and serve as a support of other chemically active molecular species. In the
area of detection and identification of volatile organic chemicals, conducting polymer sensors can be used independently for the detection and analysis of individual vapors, or in the form of a heterogeneous array for the analysis of complex mixtures. In general, when a conducting polymer is exposed to an analyte vapor, the electrical conductivity of the polymer is changed. The change is correlated to the concentration of molecules, and can be readily reversed when vapors are removed.\textsuperscript{3} In a simple yet outstanding work, Janata and co-workers combined Ppy membranes and FET devices to fabricated miniaturized solid-state gas sensors that showed rapid and high sensitivity to aliphatic alcohols at room temperature.\textsuperscript{5} These sensors displayed excellent characteristics that immediately directed numerous efforts on sensor research to employ conducting polymers.\textsuperscript{6}

The conduction mechanism of conducting polymers has been elucidated over years of research. Researchers have concluded that conducting polymers are quite different from inorganic semiconductors. The organic molecules (monomers) that constitute these polymers are oxidized to radical cations or dications and the former may interact to form dimers. Anions are incorporated into the growing polymer, interacting with various strengths with the positive charges of the polymer chain, and finally, solvent molecules may be intercalated, influencing the local electric fields. All of these factors affect the conductive properties. In their most advanced molecular designs, conducting polymers involve a functional group capable of selectively responding to a chemical or physical stimulus delivered by the external environment to a $\pi$-conjugated backbone. The conjugated backbone is capable of transporting the electrical information from the sensitive group to an external circuit for signal processing. The mechanism of charge
transport in conducting polymers is dependent upon many electronic, chemical, and structural factors. Figure 5.1 shows the valence bond structure of positively charge (p-doped) polarons and bipolarons of a doped PEDOT polymer chain with anions accepting the electrons removed from the polymer chain. The term polaron is borrowed from condensed matter physics to refer to the removal of an electron (a radical cation) (Figure 5.1a). The removal of a second electron from a polaron would generate a bipolaron (Figure 5.1b). In the field of conducting polymers, the generation of polarons and bipolarons is a redox process that is accomplished during doping with counter ions.

The analyte effect on carrier mobility in conducting polymers has been used to design highly sensitive sensors in which molecular interactions are used to trap charge carriers or inject new ones. An example of such a molecular engineering approach can be seen in the work of Swager and co-workers. They introduced receptor units to the backbone of conducting polymers that can interrupt charge-carrier transport on binding a target analyte, thereby creating polymeric, reversible, and chemoresistive sensors.\footnote{7}

Chemical and biological sensors using conducting polymers are generally formed from films of the electroactive material fabricated on a pattern of metallic or semiconductor electrodes. The goal of modifying an electrode with a conducting polymer is to improve sensitivity, to impart selectivity, and to minimize the effect of interfering reactions. Currently, nanostructured materials fabricated from conducting polymers are being investigated as discrete components in chemical sensors and electronic devices. Examples of such systems are organic vapor sensors based on single-walled CNT integrated into FET and interdigitated arrays,\footnote{8,9} polymer wire chemical sensors bridged across a microfabricated tuning fork,\footnote{10} conducting polymer nanowires
fabricated by dip-pen nanolithography across electrodes,\textsuperscript{11} and composite organic-inorganic nanowires that exhibit diode behavior.\textsuperscript{12}

Ramanathan \textit{et al.} reported the fabrication of conducting polyaniline nanowires using electrodeposition between electrodes in channels created on insulating surfaces.\textsuperscript{13} Their technique was capable of producing individually addressable nanowire sensors, with site-specific positioning, alignment, and chemical compositions. Similar work by another group at Cornell University showed polyaniline nanowires deposited on gold electrodes that exhibited a rapid and reversible resistance change upon exposure to NH\textsubscript{3} gas at concentrations as low as 0.5 ppm.\textsuperscript{14} The single-wire geometry in both studies allowed for the characterization of the nanowire material and the device response. Nevertheless, fabrication of these nanowires is limited to a few at a time and lacks reproducibility.

Previously, Tao and coworkers reported the current-voltage (I-V) characteristics of conducting polyaniline nanowires (~6 nm) fabricated by the electrochemical polymerization of aniline onto a sharp scanning tunneling microscope (STM) tip held at a small distance (20-100 nm) from a Au electrode.\textsuperscript{15} Studies of the I-V characteristics of these nanowires as a function of the electrochemical potential in electrolyte solution showed that depending on the potential, the I-V curves varied from ohmic to rectifying behavior. They estimated conductivities for the nanowire at the nanojunction to be on the order of 10-100 S/cm, almost certainly due to the alignment of polymer chains during a stretching step performed as part of the fabrication process. This ability to control the polymer’s electrical behavior supports the application of conducting polymer nanowires
not only as conductive wires for interconnections and sensors but also as active circuit elements in electronic assemblies.

Certainly, nanotubes and nanowires offer the prospect of high sensitivity and rapid detection. Nevertheless, the ability to incorporate these particles into sensor architectures is limited by the difficulty of manipulating and locating the nanostructures with respect to microelectrodes. Section 5.2.6 summarizes the efforts of a team of researchers at PSU to optimize their technology on “electric-field assisted alignment” of nanowire structures into high density arrays.\textsuperscript{16} The alignment technique allows for the study of the conducting polymer nanowires discussed in this chapter and provides the means for the instantaneous assembly of high density (heterogeneous) arrays of nanowire sensors and devices.

The polymers described in this chapter are called “electronic polymer junctions” for their potential application as discrete electronic components and nano-scale sensors. Their potential comes from their unique chemical and electronic properties and the ability of controlling such properties precisely and reproducibly. In particular, PEDOT nanowires are synthesized with a variety of counter ion species and their electronic properties are investigated as the first step toward their application as nano-scale gas sensors. The past few years have witnessed the rapid emergence of PEDOT among the most widely investigated conducting polymers owing to its many desirable properties such as its electrical conductivity (as high as ~550 S/cm), low band gap (1.5-1.7 eV), transparency in the conducting state, and high contrast in the visible region for electrochromic switching.\textsuperscript{17} Extensively studied for its electrochromic applications,\textsuperscript{18} PEDOT has recently demonstrated great potential in sensor applications.\textsuperscript{19,20} In addition,
the monomer can be modified by synthetic methods to impart high chemical selectivity and sensitivity to the polymer. Figure 5.2 shows the molecular structure of two molecules containing chemically selective moieties designed by Swager and coworkers for sensor applications, which are capable of undergoing electropolymerization as a result of the EDOT terminal units.\textsuperscript{21,22} In one example, the conductivity of the resulting polymer containing tungsten-capped calixarene was highly sensitive to the addition of $p$-xylene (Figure 5.2a). These types of model molecules containing highly selective and polymerizable units are the next step in the development of conducting polymer nanowires for chemical sensor applications.

There are only a few reports on the application of individual conducting polymer nanowires as sensors or electronic devices; moreover, there are fewer accounts that report on the synthesis of conducting PEDOT nanowires.\textsuperscript{11,23,24,25,26} The work presented in this chapter is a summary of the initial efforts in the development of conducting polymer nanowire arrays for gas sensing applications supported by the National Science Foundation. Nanowire synthesis, alignment and integration, characterization of their electrical properties, and initial sensing behavior are reported here. Much of this work has been a team effort. Collaborators are acknowledged for their specific contributions throughout the chapter. However, one major contributor and also the current lead student of this venture is Ms. Yanyan Cao, whose enthusiasm and energy continues to advance the development of conducting polymer nanowire sensors in our research team.
5.2 Experimental Methods

5.2.1 Materials

Anodic alumina membranes (Whatman Inc., NJ) containing cylindrical pores ca. 0.3 \( \mu \text{m} \) in diameter were employed as the templates for nanowire the growth of EP nanowires. 2,3-Dihydrothieno[3,4-b]-1,4-dioxin (also 3,4-ethylene dioxythiophene, EDOT), pyrrole (98+%), \( \text{SnCl}_2 \) (99+%), \( \text{AgNO}_3 \) (100%), \( \text{Na}_2\text{SO}_3 \) (99.5%), formaldehyde (37%), methanol (99.96%), \( \text{Na}_2\text{CO}_3 \) (99.5%), \( \text{N}_2\text{H}_4 \) (hydrazine) (98%), tetrabutyl ammonium (TBA) hexafluorophosphate (PF\(_6\)) (98%), poly(styrene sulfonic acid) (PSS, 18 wt.% in water), \( \text{NaH}_2\text{PO}_4 \) (99+%), ferrocene (98%), and hydrogen tetrachloroaurate (HAuCl\(_4\), 99.9%) were obtained from Aldrich. Aqueous ammonia (30%) and trifluoroacetic acid (neat) were obtained from J.T. Baker and Supelco, respectively. Aniline was purchased from Alfa Aesar, Silver (1025) and Au (Orottemp 24) electroplating solutions, and \( \text{Na}_3\text{Au(SO}_3\text{)}_2 \) (Oromerse Part B, 8 x \( 10^{-3} \) M) were obtained from Technic Inc., Cranston, RI. Acetonitrile, and methylene chloride (Sigma-Aldrich), and ultrapure water (18 M\( \Omega \)-cm) were used for the preparation of all solutions and for rinsing. Silicon substrates and Au 40-pin dual-in line (DIP) side braze packages were purchased from Addison Engineering, Inc., San Jose, CA. Interdigitated array structures where obtained from NIST.

5.2.2 Characterization

Optical imaging was achieved with an Olympus BX60M reflected-light microscope equipped with an Olympus U-CMAN-2 camera (Olympus, Japan). FE-SEM images were obtained with a JEOL 6700F scanning electron microscope. TEM images
were obtained with a JEOL 1200 EXII at 80 kV accelerating voltage. Electrochemical synthesis, characterization and manipulation were achieved using the electrochemical system described in the next section. Instrumentation and substrates for electrical characterization are described in subsequent sections.

5.2.3 Electrochemical System

All electrochemical experiments were performed using either one of two potentiostat/galvanostats (BAS 100B or a Princeton Applied Research, model 363) in a one-compartment, three-electrode cell at room temperature (Appendix A). The electrochemical cell was protected with a Teflon cap specially designed to hold the three electrodes used and to prevent the evaporation of organic solvents. A platinum foil (2 cm$^2$) served as a counter electrode. An SCE, or a silver wire served as the reference electrodes when specified. A silver wire was employed as pseudo reference electrode and was calibrated using a ferrocenium/ferrocene (Fc$^+/Fc$) couple ($E^* = 0.400$ V vs. NHE$^{27}$) as an internal standard to determine its potentials.$^{28}$ The silver wire was tested in solutions containing 0.1 M electrolyte (counter ion employed during the synthesis of PEDOT) and 0.001 M of ferrocene in nonaqueous methylene chloride. Scans were performed at broad potential windows to identify the redox couple waves. The silver wire was also calibrated by polymerizing EDOT in the presence of ferrocene on Au covered silicon substrates. Used of the formula $E^f = (E_{p(anodic)} - E_{p(cathodic)})/2$ allowed the approximation of the formal potential for the initial oxidation of EDOT of 1.7 V vs. $E^f(Fc^+/Fc)$.
A thin layer (150 nm) of sacrificial Ag was deposited on the branched side of the anodic alumina membranes by thermal evaporation to serve as the working electrode. Electrochemical deposition occurred inside the alumina membranes producing straight, monodisperse composite nanowires of desired lengths. Polymer films were grown onto gold-coated silicon substrates or on interdigitated array structures also assembled in a three-electrode cell configuration.

5.2.4 Synthesis of Electronic Polymer

Conducting polymer films were initially grown onto silicon substrates coated with a 150 nm Au layer or onto gold interdigitated arrays by potentiostatic oxidation (electropolymerization). The synthesis of conducting polymer films on gold-coated planar substrates served as a standard method for determining the most favorable conditions for polymer growth. In fact, the growth of polymer nanowires is not a straightforward task. The protocols for the synthesis of conducting polymers as established in the literature must be slightly modified (e.g. potential window, scanning rate, or monomer concentration) to promote polymer growth within the pores of anodic alumina membranes.

Polyaniline films and nanowires were both synthesized from aqueous 0.1 M aniline, 2 M HCl, and by potential sweeps between 0 to 1.2 V vs. SCE at 50 mV/s.

PEDOT films were grown by cycling the applied potential at the working electrode from 0.05 M EDOT monomer solutions and 0.1 M TBAPF$_6$ (or aqueous PSS) in methylene chloride or acetonitrile. The monomer was distilled and solutions were freshly prepared immediately prior to use. The electrochemical cell was covered with a
Teflon cap designed to hold the electrodes in the electrochemical cell and prevent rapid evaporation of the organic solvents. Potential sweeps between 0.3 V to 2.2 V vs. $E^{'}(\text{Fe}^{2+}/\text{Fe})$ at 20 mV/s yielded the best growth.

PEDOT nanowires were synthesized by the template replication method employed throughout the thesis.\textsuperscript{29} The templates used were alumina membranes (see Appendix A for additional image) characterized by having a branched side and an unbranched side that results from the anodization process employed to produce cylindrical pores.\textsuperscript{30} A sacrificial silver layer was evaporated on the branched side of 60 µm thick anodic alumina membranes containing cylindrical pores ca. 0.3 µm in diameter to serve as the working electrode. Approximately 3-4 µm of additional sacrificial silver was electrodeposited into the alumina membrane to fill the branched section of the template and prevent branched nanowire structures.

Unless otherwise noted, gold segments were grown prior to polymer deposition. (The next section describes the gold deposition in detail.) Polymer nanowires were grown from 0.05 M EDOT monomer solutions and 0.1 M counter ions in methylene chloride or acetonitrile. The electrochemical cell was scanned between 0.3 V to 2.2 V vs. $E^{'}(\text{Fe}^{2+}/\text{Fe})$ at 20 mV/s for 15 cycles to produce approximately 3 µm long segments. The alumina membranes were subsequently treated with 25% (v/v) HNO$_3$ to remove the sacrificial silver, and 0.5 M sodium hydroxide to dissolve the alumina membrane and obtain free-standing nanowires. Nanowires were rinsed approximately three times with water and three times with methanol or ethanol, and then re-suspended in methanol, ethanol, or isopropyl alcohol (IPA).
5.2.5 Synthesis of Segmented Metal/EP/metal Nanowires.

The synthesis of segmented gold/EP/gold nanowires entails a very similar process to that described in Chapter 2 for the synthesis of gold/polypyrrole/gold nanowires, and is illustrated in Figure 5.3. Electrodeposition of Au was carried out at a constant potential of –0.9 V vs. SCE using a commercial Au plating solution containing approximately 6.9% potassium aurocyanide (KAu(CN)₂). Au-containing membranes were rinsed with acetone and dried with nitrogen. Monomer solutions were freshly prepared and EP segments were electrodeposited as described in the previous section. The second Au segment was grown by several chemical deposition methods. First, a slightly modified version of the electroless deposition method described by Menon et al. was employed. In general, membranes were successively reacted at 25 °C with 0.025 M SnCl₂ and 0.07 M trifluoroacetic acid in 50:50 methanol/water (1 hr), 0.03 M AgNO₃ in 0.3 M aqueous ammonia (10 min), and a solution containing 0.008 M Na₃Au(SO₃)₂/0.129 M Na₂SO₃/0.625 M HCHO adjusted to pH 10 (12 hrs), then rinsed with copious amounts of methanol and water. Although this method proved to be successful for the synthesis of Au/Ppy/Au nanowires, there were critical problems with the electroless deposition onto PEDOT segments. These problems are addressed with appropriate experimental details in section 5.3.3.

5.2.6 Electric Field Alignment of Nanowires

Electric field assembly is becoming increasingly important to align large number of nanoparticles rapidly and precisely into lithographically defined electrodes. Researchers at PSU have perfected this technique, in particular for the alignment of
anisotropic nanoparticles and the creation of high density arrays of individual nanowires that can be individually and electronically addressed.

An alternating current (AC) electric field was used to align anisotropic nanoparticles such as the nanowires described throughout this thesis. Electrically isolated electrodes were photolithographically patterned on silicon wafers as depicted in Figure 5.4. First, bus bars were patterned by 50 nm Ti/150 nm Au on silicon dioxide (SiO$_2$). Next, a silicon nitride (Si$_3$N$_4$) dielectric layer was deposited by chemical vapor deposition (CVD) to isolate the bus bars, and alignment electrodes were finally deposited. The alignment electrodes have a separation of 3.5 to 4 µm. Typically, an array of 100 x 100 alignment electrodes are grouped in devices called alignment cells.

An AC field of 1 kHz and a voltage of 15 V$_{rms}$ were typically applied to the bus bars, and 5 µL of a solution of IPA containing $10^5$ nanowires was placed over each alignment cell. Capacitive coupling between the bus bars and the alignment electrodes produces an electric field that creates a charge separation on the nanowires, orienting and moving them in the direction of increasing field. Composite gold/conducting polymer/gold nanowires were successfully aligned for the characterization of their electrical and sensor properties.

### 5.2.7 Electrical Measurements

Electrical measurements were performed at ambient temperature with a HP 4176B Precision Semiconductor Parameter Analyzer coupled to a probe station. The electrical properties of gold/PEDOT/gold nanowires with polymer segments ~ 4 µm in
length were measured using the alignment electrodes described in the previous section designed to allow integration of large area pads for mechanical probing (Figure 5.5).

5.2.8 Nanowire Integration

Chemical microsensor arrays are usually implemented in silicon technology. The capability to fabricate a very large number of dedicated sensing elements exceeds the ability to incorporate the number of orthogonal chemically sensitive layers by several orders of magnitude. Also, the ability to extract the raw outputs from the individual sensing elements present a design and fabrication challenge. Integrating segmented gold/conducting polymer/gold nanowires into large arrays of lithographically defined electrodes is a viable approach to increase orthogonality, redundancy, and selectivity in array sensors.

The integration of nanowires containing electronic polymer junctions is a way to connect individual composite nanowires to the macroscale world for characterization and further testing. Although electrical characterization can be accomplished by use of a probe station, this method is not feasible for testing the sensory response of many nanowires in a controlled environment, or for future applications of nanowire array sensors.

Nanowires were integrated with CMOS technology by combining the technology developed for the electrofluidic assembly of nanoscale particles, and advanced microelectronic packaging. Established technology by our collaborator, Dr. Steve Semancik (Chemical Microsensor Project Leader), at NIST, lead us to adopt the gold-plated 40-pin DIP side braze package as the packaging device to contain the nanowire
arrays. The previously designed array structure of alignment electrodes was reconfigured (with the help of Haripryia Prakassan, a graduate student in the Electrical Engineering Department at PSU) to contain 20 pairs of electrodes individually interconnected to large (100 µm x 100 µm) area pads. The 20 pairs of alignment electrodes were fabricated by standard CMOS process and are referred to as alignment chips. The new chip contained the same number of layers as the previously described alignment structure (section 5.2.6) and can be fabricated by the same step by step process illustrated in Figure 5.4. The total area was designed to be 8 mm x 8 mm due to packaging restrictions and is depicted in Figure 5.5.

Chips were assembled in standard 40-pin DIP packages by wire bonding each alignment electrode to its own pin (see Figure 5.6c). The final encapsulation was completed by a chemically resistant high grade encapsulant when chemical post processing steps may be required. The encapsulation step provides chemical resistance and general protection of the electrical interconnects that bridge to individual nanowires. Although these attributes are less important for gas sensing applications, they provide the opportunity for the modification of conducting polymers by solution chemistry.

Figure 5.6 summarizes the nanowire integration process. First, nanowires were aligned into alignment chips by applying the appropriate parameters to the bus bar electrodes as described in the previous section. Second, the chip was attached to the center of the 40-pin DIP package and each individual electrode was wire bonded to a corresponding pad in the package. Third, all wire leads and wire bonding areas were encapsulated and isolated. This process can be applied to any type of electrode microstructure that may need integration to the macro scale. Finally, the DIP package was
inserted into a corresponding socket where an electrical signal can be applied or measured at each individual electrode connecting to a nanowire. In the future, nanowire arrays will include the ability to control the temperature locally by integrating the pre-existing micro-hotplate\textsuperscript{33} technology developed at NIST to the alignment technology developed at PSU.

IDA electrodes coated with conducting polymers are a convenient and popular conductometric format for sensor devices, and offer a platform for polymer characterization. Collaborators at NIST used the IDA platform as shown in Figure 5.7 to study the potential of conducting polymer films, such as polyaniline,\textsuperscript{34} in gas sensing applications. IDAs were employed as a standard platform for the synthesis and characterization of sensing properties of novel conducting polymer systems. This allowed rapid assessment of the potential of certain polymer/counter ion systems before synthesizing their nanowire counterpart.

5.2.9 Gas Flow/Dilution System

A gas dilution system with capabilities to deliver concentrations as low as a few ppm (depending on gas and temperature) to a 1 cm\textsuperscript{3} chamber containing sensor elements, has been developed for the testing of different kinds of nanostructured materials, including conducting polymer nanowires. The system is analogous to the pre-existing state-of-the-art sensing technology available in the Process Measurement Division at NIST. Appendix B shows a diagram and photograph of the dilution system designed by Dr. Steve Semancik (NIST), Professor Theresa Mayer and Dr. Mohammad Islam (PSU, electrical engineering), and Professor Thomas Mallouk (PSU, chemistry), and assembled
by Applied Energy Systems, Malvern, PA. In addition, Mr. Richard Geiger (PSU, electrical engineering) and Ms. Yanyan Cao (PSU, chemistry), continue to expand the system’s capabilities to include controlled humidity, flow of low vapor pressure organic solvents, temperature control at the sensor elements, and a variety of sensor interfaces.

5.3 Results and Discussion

5.3.1 Nanowire Test Structure

The use of IDAs has been extended to characterize nanowires and nanotubes due to its simplicity and availability.\textsuperscript{9,35,36} Figure 5.8 shows an IDA with gold nanowires aligned by the field-assisted alignment approach. Electrical characterization by this platform lacks quantitative value since nanowires are aligned randomly and contact resistance between IDAs and nanowires varies for each nanowire. Table 5.2 shows the number of Au nanowires aligned in a single gold-IDA vs. overall resistance. Au nanowires were selected to minimize the contact resistance between the nanowires and IDA. The highly scattered data demonstrate the lack of proportionality in the measurement even for a system comprising gold to gold contacts. This is a result of inconsistencies in the contact among nanowires and IDA. Table 5.2 also shows the resistance of the same systems after electrochemical deposition of gold was performed on the same samples. As the contact between particles and IDA improved, resistance values became inversely proportional to the number of Au nanowires (surface area) but reached a constant value. Alignment of several (~10) nanowires shorted the IDA and no resistance was registered. Although the IDA is a great platform for the characterization
of films of many different materials, nanowires must be individually addressed to truly exploit the superior properties and performance of these particles.

5.3.2 Polyaniline: A Model Conducting Polymer for Sensing Applications

In the last decade, polyaniline was widely studied because of its environmental stability, low cost, and ease of synthesis. Its large range of conductivity vs. doping level allowed its specific application in light emitting diodes, batteries, antistatic coatings, gas sensors, and more. It is one of the so-called doped polymers, in which conductivity results from a process of partial oxidation or reduction. Polyaniline compounds can be designed to achieve the required conductivity for a given application. The resultant blends can be as conductive as doped silicon and germanium or as insulating as glass. Since polyaniline has been extensively studied in thin film gas sensing applications,\textsuperscript{5,13-15,34,37} it was chosen as a model conducting polymer during the initial phase of this project.

Polyaniline films were synthesized in bi-metallic IDA structures from 0.1 M aniline in 2 M HCl, and potential sweeps between 0 to 1.2 V vs. SCE at 50 mV/s (Figure 5.9a). Interestingly, platinum surfaces showed better nucleation of polyaniline than Au electrodes resulting in (more desirable) higher surface area. Figure 5.9b and Figure 5.9c show early and final stages of the polyaniline electropolymerization on Pt electrodes. The green color in the optical micrograph results from the emeraldine salt form of the polymer as a result of the acid doping. It must be noted that fibrils formed (Figure 5.9b) by direct polymerization on Pt-IDA have a smaller diameter (<10 nm) than nanowires of the same kind discussed in this chapter. Figure 5.10a shows the ease of controlling the doping levels of polyaniline films by exposure to hydrochloride acid solutions of varying
pH values for 30 minutes and drying periods of 24 hours, and Figure 5.10b shows the rapid recovery in conductivity upon exposure to acid. The resistance increased linearly with increasing pH as expected. After 24 hours of exposure to ultrapure water (18MΩ-cm), the polyaniline film turned to a dark brown color and the value of resistance could not be measured with conventional meters. Nevertheless, the conductivity quickly recovered after exposure of the film to 2 M HCl for less than 20 minutes (Figure 5.10b).

Polyaniline nanowires were synthesized on alumina membranes containing gold segments using the same parameters for the synthesis of thin films described above. Figure 5.12 shows the cyclic voltammogram of the synthesis of polyaniline nanowires inside the membrane; also characteristic of the synthesis of polyaniline films onto gold electrodes in the presence of HCl. Proton doping is only obvious on single scans in this voltammogram, yet it occurred at ~0.1 V vs. SCE. The uptake of chloride ions occurred at ~0.25 V vs. SCE. Janata and coworkers investigated the origins of these peaks and determined that the very intense cathodic peak at ~0.5 V may result from the reduction of chloroaurate ions formed during the oxidation wave at 0.85 V. Chloroaurate ions can be intercalated into the polymer matrix as a counter ion. According to their studies, chloroaurate ions are reduced to form metallic Au clusters on the reverse scan as determined by X-ray photoelectron spectroscopy (XPS).

A TEM micrograph of a single polyaniline nanowire (Figure 5.13) reveals a very unusual surface morphology compared to other polymer nanowires. These apparent features could be the result of exposure of the polymer to NaOH when dissolving the membrane. In fact, polyaniline also dissolves slowly in NaOH solutions, creating the globular morphology seen in Figure 5.13. Moreover, the contrast between the gold
segments and the globular features suggest that these may be gold clusters in the polymer as reported by Janata.

Au/polyaniline nanowires were aligned on IDAs (the only available platform at the time of the study) to study their electrical properties as a function of doping levels. IDAs containing aligned polyaniline nanowires were rinsed with a powerful stream of water to remove excess nanowires and were annealed at 50 °C. Contact to individual nanowires was achieved by mechanically removing other nanowires from the IDAs using glass micropipets as a tool. This crude method resulted in many broken and scratched surfaces and poor measurements. However, Table 5.3 shows the data recovered from that period of struggle to find proper single wire characterization technology. The average conductivity for polyaniline nanowires (~250 nm in diameter and 7 µm long) calculated from the values in Table 5.3 is 16 ± 8 S/cm. Such a high value of conductivity may be due to the possible gold content in these nanowires.

It is probable that this morphology contributed to the poor physical contact between nanowires and electrodes (from the scattered data), and that the metallic composition contributed at the same time to the high conductivity observed (high values). Additionally, the mechanical stability of polyaniline nanowires was extremely poor due to structural damage induced by exposure to NaOH. Unfortunately, there is no additional data in this study that can provide further chemical and structural evidence to support these conclusions. Polyaniline nanowires synthesized as described in this chapter must be studied in more detail to determine their true chemical nature before their application. It must be mentioned that electrical measurements on individually addressable polyaniline nanowires (~100 nm in diameter and ~4 µm long) have been recently
reported. Those nanowires were electropolymerized directly between micro electrodes to eliminate post processing and allow the characterization of polyaniline nanowires. However, such a fabrication method is not suitable for high density nanowire arrays since the structures generated are not reproducible, and the complex growth process restricts the electropolymerization to one pair of electrodes at a time.

5.3.3 Poly(3,4-Ethylene Dioxythiophene) (PEDOT) Nanowires: Setting the Standard for New Nanosensors

5.3.3.1 Synthesis of Segmented Gold/PEDOT/gold Nanowires

In the case of polymer films, electrical contact is achieved by depositing the polymer on metallic, carbon-based, or semiconductor electrodes or substrates. Adhesion to this substrate must be maintained to ensure stability of the system. Naturally, the ideal conducting polymer nanowire model defined throughout the thesis is a single polymer segment capped by metallic gold ends to minimize contact resistance between electrodes (Figure 1.3). Likewise, it is important to maximize adhesion of the different segments in composite nanowires to ensure proper performance and eliminate unpredictable behaviors due to inadequate junctions.

Gold/PEDOT nanowires were grown in alumina templates by potential cycling from 0.05 M EDOT, 0.1 M TBA PF$_6$ in methylene chloride as shown in Figure 5.14. The cathodic current increase at the highest potential corresponds to the oxidation of the monomer and formation of PEDOT. The oxidation and reduction of PEDOT were revealed by the cathodic peak centered at 1.6 V vs. Ag wire (1.3 V vs. $E^\circ(\text{Fc}^+/\text{Fc})$) and a smaller anodic peak at 0.8 V vs. Ag wire (0.5 V vs. $E^\circ(\text{Fc}^+/\text{Fc})$), respectively. These
peaks are in good agreement with studies on the spectro-electrochemical behavior of PEDOT films reported by Kvarnström and coworkers.\textsuperscript{38}

Gold/PEDOT nanowires were removed from the template and examples of the resulting structure and morphology can be seen in Figure 5.15. TEM micrographs show PEDOT segments with apparent open ends. Previous work on PEDOT nanowires synthesized electrochemically by the template replication method also showed nanowires with open ends that exhibited field-emission (FE) properties. Further discussion comparing PEDOT nanowires with FE properties and the nanowires developed in this chapter will follow in the next section. Figure 5.16 shows typical electron micrographs of electrodeposited gold/PEDOT:TBAPF$_6$ junctions. These junctions were exceedingly stable, and a $\sim$25\% decrease in the diameter of the polymer segment was observed. The decrease in diameter became problematic during the deposition of subsequent materials as observed in Figure 5.17. Au growth could be observed on the surface of PEDOT nanowires throughout the entire length of the polymer segment. Electrochemical deposition of Au over PEDOT segments resulted in metal growth connecting both ends of the nanowires, as observed in Chapter 2 for the case of polypyrrole nanowires.

To achieve top metallic contacts on PEDOT segments for the fabrication of segmented Au/PEDOT/Au nanowires, an electroless gold deposition process was employed, similar to the approach taken in Chapter 2. (The next section on electrical properties of nanowires discusses the importance of stable metallic contacts at each end of nanowires containing the electronic polymer junction for the characterization and performance of these nanowire devices.) Figure 5.18 shows various examples of electroless deposition of gold onto PEDOT:TBAPF$_6$ nanowire segments from a solution
containing 0.008 M Na₃Au(SO₃)₂/ 0.129 M Na₂SO₃/ 0.625 M HCHO adjusted to pH 10. Gold deposition occurred throughout the entire length of the PEDOT:TBAPF₆ segment. Moreover, the micrographs in Figure 5.18 suggest that nucleation of gold occurred readily, producing large amounts of nanoscopic gold particles. This resulted in mechanically unstable gold segments that would break apart upon removal of the membrane. These results contrast with those on the electroless deposition of gold onto Ppy nanowires discussed in Chapter 2. Evidently, there are many parameters that influence the chemical processes involved in the electroless deposition process, and the chemical interactions among materials. The chemical nature of the conducting polymer, dopants, and gold precursors must be considered in order to prevent interpenetration of metal into the polymer.

Ms. Yanyan Cao developed a PEDOT:polystyrene sulfonic acid (PSS) polymer nanowire structure that eliminated metal interpenetration into the polymer segments. PEDOT:PSS nanowire segments were synthesized by electrochemical deposition from a solution containing 0.05 M EDOT, 0.1 M PSS in 1:1 ratio acetonitrile: water. The use of a large molecule such as PSS as a counter ion during the electropolymerization of PEDOT, prevented the ion exchange with the different ionic species used in the electroless deposition process. It is believed that PSS cannot diffuse out of the polymer matrix due to its large size, hence Au deposition is restricted to the surface of the polymer segment.

Another approach to the fabrication of well defined polymer-Au junctions is to design electroless deposition processes with low affinity to the polymer matrix (to prevent interpenetration), and appropriate nucleation and growth rates to achieve a solid
metal segment. Variables that can control the characteristics of the metal deposits are: chemical nature of the ionic species in the electroless bath, catalyst, pH, temperature and even the chemical nature of the counter ion present in the polymer matrix. Consequently, with every new conducting polymer system, a new electroless deposition process must be devised to achieve proper polymer-metal junctions. Figure 5.19 shows an example of a PEDOT-Au junction deposited by changing the nature of the electroless bath. The new bath consisted of an aqueous solution of $0.25 \text{ M} \ NaH_2\text{PO}_4$, $1.25 \text{ M} \ HCHO$, and $0.0025 \text{ M} \ HAu\text{Cl}_4$, at pH 5 and yielded more stable and dense PEDOT-Au junctions with a gold penetration depth of approximately 600 nm.

### 5.3.3.2 Electrical Characterization of Nanowires

Nanowires (~300 nm in diameter and 4-6 µm long) were aligned on alignment cells and the I-V characteristics of individual nanowires were recorded. Electrical properties of Au/PEDOT:PSS nanowires and Au/PEDOT:PSS/Au nanowires are compared in Figure 5.20a. The PEDOT segments in both types of nanowires were synthesized by using the same parameters, thus the difference in the electrical response is due to the absence of a gold contact in Au/PEDOT nanowires. Nanowires containing metal contacts at each end exhibited an ohmic response. The current relationship with the applied potential is nearly linear as expected for the metallic phase of the PEDOT nanowires; however there is a slight deviation from simple ohmic behavior, particularly with increasing voltage (Figure 5.20b). The deviation may be due to an electric-field-induced change in the conductivity, which has been observed in bulk materials. Nanowires with only one metallic end displayed poor charge transfer at the junction.
between the polymer end and the gold electrode, and this had a tremendous negative impact on the performance of the nanowire. Measurements such as those in Figure 5.20a made clear the need for metal junctions at each end of polymer nanowires to ensure reproducible behavior and performance, and demanded the development of various protocols for the chemical deposition of gold ends onto PEDOT segments as discussed in the preceding section (section 5.3.3.1).

The electrical conductivity of Au/PEDOT:PSS/Au (developed by Ms. Yanyan Cao) and Au/PEDOT:TBAPF$_6$/Au nanowires was determined to be $5.21 \pm 1.5$ and $0.3 \pm 0.07$ S/cm$^2$, respectively. All the values of conductivities mentioned here are consistent with reported values for PEDOT films. Values of conductivity for PDOT:PSS films range from 0.1 to 20 S/cm. The range in the reported conductivities of conducting polymers is a result of variations in structure and chemical composition from differences in the synthetic method from one study to another. The consistency of these values shows that the synthetic method employed to fabricate Au/PEDOT/Au nanowires produces nanowires with reproducible electrical characteristics that can be engineered to meet a specific application.

The electrical properties of conducting PEDOT nanowires have also been studied by Kim and coworkers. Their study concluded that electrical properties of PEDOT nanowires (~200 nm in diameter and ~40 µm long) doped with dodecylbenzenesulfonic acid were dependent on synthetic conditions such as doping level, polymerization time, and applied current; and the measured conductivity of a single wire was $\sim 3.4 \times 10^{-3}$ S/cm. This lower value of conductivity could result from their aqueous synthetic conditions and structural defects arising from the large length scale. The conductivity of
another kind of PEDOT:PSS nanowire (diameter <10nm) has been measured by manipulation with AFM into nanoelectrodes. The values of conductivity reported were 0.6 and 0.09 S/cm for two single nanowires.

The doping-charge dependence of conductivity in conducting polymers became important when polypyrrole doped with perchlorate\textsuperscript{41} or tetrafluoroborate\textsuperscript{42} salts showed a linear increase in conductivity with charge until it reached a plateau. The independence of conductivity with charge at high levels of doping was explained as free charges hopping within a narrow potential range with constant density of states.\textsuperscript{43} The resistance of each type of nanowire can be modulated by adjusting the doping level of the conducting polymer using mild chemical oxidizing agents (FeCl\textsubscript{3}, NO\textsuperscript{+}PF\textsubscript{6}\textsuperscript{−}, I\textsubscript{2}, etc.), by using chemical reducing agents such as ammonia or hydrazine, or electrochemically using controlled potentials. The conductivity is caused by the combined effect of both the high mobility of charge carriers along the polymer chain and a high rate of electron exchange between the conjugated chains.\textsuperscript{44} The electronic conductivity is normally higher than the ionic conductivity; therefore the rate of electrochemical oxidation/reduction of the polymer will be determined by the rate of counterion movement required for electroneutrality between the electronic and ionic charges. For example, composite Ppy nanowires doped with carbon nanotubes (CNT) showed superior electronic properties compared to the chloride doped analog. Such enhancement was attributed to the contribution in conductivity from the CNT compared to non-conducting dopants. While many models have been proposed for conduction in conducting polymers, conduction has been found to be a very complex phenomenon, and to date no single model is comprehensively accurate.
Reduction of the polymer by chemical reducing agents involves a change in the electronic conductivity induced by nucleophilic attack on the polymer conjugated backbone. Figure 5.21 shows the I-V characteristics of an Au/PEDOT/Au nanowire (~300nm in diameter and 4 µm long) before and after exposure to an aqueous solution of 1% hydrazine for 30 minutes. Hydrazine has a very strong nucleophilicity that makes it even more reactive than ammonia to conjugated systems. It was employed to reduce PEDOT and to control the electrical conductivity. The conductivity was reduced by three orders of magnitude and can be further reduced by longer exposure. The resulting conductivity of the reduced PEDOT segment retained its linear characteristics, which confirmed the ability to modulate the inherent conductivity of PEDOT nanowires and still maintain the electronic integrity of the material.

The electrical behavior of conducting polymers is an important parameter for the application of these materials as electronic sensor elements given that models can be established that predict the influence of an analyte molecule on conductivity. However, it must be noted that to obtain a complete picture of the behavior and response of a conducting polymer, it does not suffice to merely measure DC conductivities. Rather, the characterization of the entire range of conduction behavior (conductivity as a function of temperature, frequency, magnetic field, processing and composition) can reveal unique responses to analytes not evident from DC measurements.

5.3.1 Nanowire Sensor

The synthesis, integration and characterization of nanowires containing electronic polymer junctions have been successfully accomplished, and outline a unique
methodology for fabricating arrays of nanostructured sensors and devices. There are several advantages to the methods established in this thesis. First, resistivity of multiple and individual nanowires can be measured precisely with standard instrumentation. Second, conducting polymers are generally more sensitive to environmental perturbation than other sensing materials because of their inherent transport properties (electrical conduction mechanism and energy migration). Moreover, conducting polymer nanostructures such as nanowires, nanofibers, and nanotubes have a high surface area as compared to their typical bulk counterpart. Since a large amount of charge can traverse the nanowires while analyte molecules modify the transport, a single analyte binding event could affect the transport of many electrons.

Research on the development of sensor systems using PEDOT nanostructures is scarce, and there are only a few reports available that explore the potential of PEDOT in general. In one example, sensors based on multi-layers of PEDOT nanorods (synthesized by a micelle-mediated interfacial polymerization technique) on IDA, have shown measurable response to NH$_3$ and HCl vapor concentrations as low as 10 ppm and 5 ppm, respectively.\textsuperscript{20}

Other work on PEDOT films by collaborators at NIST (not published) has shown great sensitivity to water vapor. With these results in mind, Au/PEDOT:PSS/Au nanowires were integrated into 40-pin DIP packages and were tested in the chemical sensing facilities at NIST. Prior to exposure of analyte gases, the I-V characteristics of the integrated nanowires were measured to confirm linear behavior, the chamber was purged with dry nitrogen, and the electrical response of nanowires sensors was recorded at constant voltage (normally, 1 V). In most cases, nanowires exhibited a stable, linear
response that made them suitable for sensor testing. In general, nanowires that do not show linear and low noise response were ignored; nevertheless, the integration process outlined in this chapter has been improved to eliminate physical instabilities. Figure 5.22 shows the response of a single Au/PEDOT:PSS/Au nanowire exposed to water vapor. Exposure to 25 ppm of water vapor did not yield a measurable current change. A detectable change in conductance was observed at concentrations higher than 250 ppm. The poor response to water vapor was attributed to the long-term environmental stability of PEDOT:PSS at room temperature\textsuperscript{45} In fact, PEDOT:PSS stability has made it the most widely used conducting polymer as an electrode material in organic devices such as light-emitting diodes. Its stability results from PEDOT:PSS resistance to chemical degradation, hence, exhibiting little perturbation of its electronic configuration. Figure 5.23 shows the response of the same kind of Au/PEDOT:PSS/Au nanowire upon exposure to very reactive NH\textsubscript{3} gas. Although the nanowire response followed the expected decrease in conductivity (~90% decrease at saturation), it must be noted that saturation was reached after ~40 minutes, and recovery of less than 80% of the original conductance was on the order of days. The kinetics of ammonia desorption (t > 9.5 hours) were modeled using a model for the sorption and desorption of gases in polymer films that assumes that the diffusion of gases is due to transport through the polymer matrix.\textsuperscript{46} A diffusion equation for ammonia into PEDTO nanowires is derived as:

\[ \frac{I_v}{I_b} = \frac{4}{\ell} \left[ \frac{D t}{\pi} \right]^{1/2} \]  

(5.1)
where $I_t$ is the current passing though the nanowire at time $t$, $I_b$ is the new baseline current after purging for 3 hours, $\ell$ is the nanowire radius, and $D$ is the effective diffusion coefficient. Figure 5.23 shows the best fit of Equation 5.1 to the experimental data during the first hour of purging the sensing chamber with nitrogen gas after exposure to ammonia, and in this region $D$ was determine to be $7.1 \times 10^{-15}$ cm$^2$/s. This value is small compared to typical diffusion coefficients of small molecules in polymers ($10^{-10}$ to $10^{-8}$ cm$^2$/s). The slow response was indicative of slow diffusion of the NH$_3$ molecules into the polymer and is related to the highly dense structure of electrodeposited nanowires.

Previous work on polyaniline/polyethylene oxide nanowires showed a diameter-dependent behavior of their response to NH$_3$. By using a scanned-tip electrospinning method for depositing isolated and oriented polymeric nanowires, they created individual polyaniline/polyethylene oxide nanowire sensors that can detect NH$_3$ gas at concentrations as low as 0.5 ppm with rapid response and recovery time. The results indicate that the wire diameter affects the response time of the sensor, with the smaller-diameter wires having a faster response associated with the more rapid diffusion of gas molecules through the wire. PEDOT nanowires presented in this chapter have a diameter of approximately 250-300 nm, and showed extremely slow diffusion. The porosity of electronic polymer nanowires must be increased to increase diffusion rate. Further studies (not presented in this chapter) confirmed that the main cause of slow diffusion in these nanowires is the highly dense morphology of nanowires that results from the synthetic condition and chemical nature of dopant. Research on different kinds of conducting polymer nanowires has shown rapid response and recovery upon exposure to vapors of organic solvents.\textsuperscript{48}
5.4 Conclusions

This chapter demonstrates a new approach to fabricating self-assembled polymeric electronic devices. The electric-field assisted alignment approach to integrating individual nanowires with CMOS circuitry will be employed to position different kinds of conducting polymer nanowires precisely in order to fabricate heterogeneous high density arrays of nanowire sensors. Although the methodologies outlined here were focused on the creation of individual nanowire gas sensors, the same approach can be used to interface other kinds of nanoparticles to microfabricated electrodes, or electronic devices can clearly be used for other systems such as switches, transistors, displays, or memory elements. This represents a possible manufacturing approach, utilizing the best aspects and diversity of polymers and self-assembled materials combined with the best aspects of microelectronics technology.

A few reports have confirmed the great potential of conducting polymer materials and nanowire architecture as chemical sensors, and their contributions to the field are extremely valuable to this project. However, integration of nanostructures into practical devices is lacking in most scientific reports. There are many areas of research (synthesis, integration, chemical, structural and electrical properties, metrology, prototyping, etc.) that must be investigated in order to realize a nanowire chemical sensor array. Moreover, each area impacts the others, and studies should be coordinated to effectively develop these sensors. The chemical sensor team at PSU is working simultaneously on every aspect of the development of nanowire sensor arrays, including the architectural and classification algorithms to be employed in data processing. This chapter only
documents the initial efforts in this scientific endeavor and a more complete study representing the group effort on sensor array fabrication and characterization will be published soon.
Table 5.1: Conducting polymers of high value in the semiconductor and electronic markets.

<table>
<thead>
<tr>
<th>Conducting Polymer</th>
<th>Commercial Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly (ethylene dioxythiophene: Poly(styrene sulfonate) (PEDOT:PSS))</td>
<td>Electrode in electroluminescent lamps.</td>
</tr>
<tr>
<td>Polyaniline</td>
<td>Coating for carbon powder. Chemoresistive sensors: gas/vapor, electronic noses, chemical and biosensors. Planarization and hole transport material for OLEDs/PLEDs.</td>
</tr>
<tr>
<td>Poly(phenylene vinylene)</td>
<td>Light emission applications.</td>
</tr>
<tr>
<td>Polyarylene</td>
<td>Light emission applications.</td>
</tr>
</tbody>
</table>
Table 5.2: Number of Au nanowires aligned in a single IDA vs. resistance before and after electroplating gold on the electrodes.

<table>
<thead>
<tr>
<th># Au Nanowires Aligned in Au-IDA</th>
<th>Resistance of IDA (ohms)</th>
<th>Resistance of IDA after Au electrodeposition (Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18 ± 6</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>16 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>13 ± 5</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 5.3: Resistance of single polyaniline nanowires aligned in IDA.

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Resistance (MΩ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.113</td>
</tr>
<tr>
<td>2</td>
<td>0.076</td>
</tr>
<tr>
<td>3</td>
<td>0.053</td>
</tr>
<tr>
<td>4</td>
<td>0.230</td>
</tr>
<tr>
<td>5</td>
<td>0.122</td>
</tr>
<tr>
<td>6</td>
<td>0.098</td>
</tr>
<tr>
<td>7</td>
<td>0.055</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>0.106 ± 0.06</strong></td>
</tr>
</tbody>
</table>
5.5 References


9 Li, J.; Lu, Y.; Ye, Q.; Cinke, M.; Han, J.; Meyyappan, M. *Nano Lett.* **2003**, 3, 929.


Figure 5.1: Structure of (a) polarons and (b) bipolarons in PEDOT.
Figure 5.2: EDOT-based conducting polymer units developed by Swager and coworkers. (a) Tungsten (IV)-capped calixarene monomer (R= adamantyl group imparts specific recognition to xylene). (b) Conducting polymer sensitive to nitric oxide when M= cobalt.
Gold layer is elongated by electrochemical deposition of gold.

Silver is dissolved in 25% v/v HNO₃. The alumina template is dissolved in 0.5 M NaOH.

Gold is electrolessly deposited.

3 µm of silver are electroplated at 0.5 mA/cm².

1 µm of gold is electroplated at 0.5 mA/cm².

PEDOT is electropolymerized at 0.3 V to 2.2 V vs. E(Fc+/Fc).

Silver is dissolved in 25% v/v HNO₃. The alumina template is dissolved in 0.5 M NaOH.

Figure 5.3: Schematic diagram of the synthesis of segmented gold/PEDOT/gold nanowires.
Figure: 5.4: Schematic diagram of the photolithographic process for the fabrication of electrically isolated electrodes used in electric-field-assisted assembly of nanowires. (a) Au or TiPd bus bars are patterned on Si/SiO$_2$ substrates. (b) A dielectric layer (Si$_3$N$_4$) is deposited. (c) Au alignment electrodes are patterned onto the dielectric layer. (d) Capacitive coupling between bus bars and top electrodes induces nanowire alignment. (e) The aligned nanowires cut off capacitive coupling.
Figure 5.5: New alignment electrode array containing 20 pairs of electrodes individually interconnected to large (100 µm x 100 µm) area pads. The bus bars (purple) are buried under a silicon nitride dielectric layer, inducing a capacitance effect on the top, alignment electrodes shown in green. The optical image shows large area pads designed for additional mechanical probing.
Figure 5.6: Summary of the nanowire integration process: (a) nanowires are aligned by capacitive coupling between the bus bars and the alignment electrode that produces an electric field; (b) alignment chips are wire-bonded to a 40-pin DIP package; (c) pins in the DIP package are connected to external instrumentation.
Figure 5.7: (a) 100 µm x 100 µm interdigitated array (IDA) structures. (b) 100 µm x 100 µm IDA floating structure on microheaters.
Figure 5.8: Gold nanowires aligned on Au-IDA by cycling the potential ±10 V.
Figure 5.9: (a) SEM of polyaniline on bi-metallic IDA. (b) polyaniline on Pt-IDA at an early stage (5 cycles). (c) SEM and optical image of esmeraldine salt form of polyaniline on Pt-IDA after 20 cycles.
Figure 5.10: (a) Polyaniline film exposed to HCl of different pH values. (b) Dedoped polyaniline film exposed to 2 M HCl.
Figure 5.11: Electrical response of polyaniline on IDA exposed to 1% NH$_3$ at the chemical sensing facilities at PSU (courtesy of Ms. Yanyan Cao).
Figure 5.12: CV of the synthesis of polyaniline nanowires from 0.1 M aniline in 2 M HCl, and potential sweeps between 0 to 0.85 V vs. SCE at 50 mV/s.
Figure 5.13: TEM image of Au/polyaniline nanowires. The red box shows the polyaniline-Au junction.
Figure 5.14: CV of the synthesis of PEDOT nanowires from 0.05 M EDOT, 0.1 M TBA PF$_6$ in methylene chloride; from 0 to 2500 mV vs. Ag wire, 100 mV/s.
Figure 5.15: TEM micrographs of PEDOT nanowire showing apparent open ends.
Figure 5.16: Typical electron micrographs of electrodeposited gold/PEDOT:TBAPF$_6$ junctions.
Figure 5.17: TEM images showing electrodeposited gold (dark color) onto PEDOT (light color) segments (a) near the gold-polymer junction of the nanowires, and (b) at the polymer end.
Figure 5.18: TEM images showing the electroless deposition of gold on PEDOT ends at (a) early stage (~3 hours), and (b) after 24 hours. (c) Gold growth inside PEDOT.
Figure 5.19: TEM images of an improved (a) PEDOT-electroless gold junction, and (b) solid electroless gold segment.
Figure 5.20: (a) Typical I-V characteristics of Au/PEDOT (red) and Au/PEDOT/Au (black) nanowires. (b) I-V of Au/PEDOT/Au nanowire (black) showing a slight deviation from ohmic (red) behavior.
Figure 5.21: I-V of Au/PEDOT/Au nanowire before (black) and after (red) exposure to aqueous hydrazine for 30 minutes. The inset shows an expanded portion of the I-V characteristics from 0-3 V of Au/PEDOT/Au nanowire after exposure to hydrazine.
Figure 5.22: Electrical response of a single Au/PEDOT:PSS/Au nanowire exposed to water vapor in the chemical sensing facilities at NIST: (a) 25 ppm, (b) 250 ppm.
Figure 5.23: Electrical response of a single Au/PEDOT:PSS/Au nanowire exposed to 1% NH₃ at the chemical sensing facilities at PSU. Bottom graph shows the best fit to Equation 5.1 for the first hour of purging ammonia gas with nitrogen gas.
APPENDIX A

ELECTROCHEMICAL CELL

Anodized Alumina Membrane

Cell for Aqueous Electrochemistry

Cell for Non-aqueous Electrochemistry
(a) Top view electron micrograph of the un-branched side of a 300 nm pore diameter alumina membrane. (b) Field-emission electron micrograph of anodized alumina membrane fabricated in the Mallouk research group by Mr. Brad A. Lewis.
Schematic diagram of a one-compartment, three-electrode electrochemical cell; the counter electrode is a Pt coil or foil placed parallel to the working electrode (alumina membrane or silicon substrate).

Schematic diagram of a one-compartment, three-electrode electrochemical cell covered with a Teflon cap to minimize loss of solvent; the counter electrode is a Pt coil or foil placed parallel to the working electrode, the reference electrode is a Ag wire for synthesis in organic solvents.
APPENDIX B

NANOWIRE INTEGRATION AND SENSING

L-Edit Illustration of Two Types of Alignment Cells

Gas/flow Dilution Facilities at PSU
L-Edit Illustration of Two Types of Alignment Cells
Gas/flow Dilution Facilities at PSU
APPENDIX C

PROTEIN ANALYSIS WITH WESTERN BLOT

Step by Step Procedure

Sample
1) **Gel electrophoresis**: The first step is gel electrophoresis. The proteins of the sample are separated according to molecular weight on a gel, usually sodium dodecyl (lauryl) sulfate-polyacrylamide gel. Usually the gel has several lanes so that several samples can be tested simultaneously. However, it is also possible to use a 2-D gel that spreads the proteins from a single sample out in two dimensions; proteins are separated according to isoelectric point in the first dimension, and according to their molecular weight in the second dimension.

2) **Transfer**: Electrophoresis gels are thick and fragile, and as a result, it is difficult to carry out all the steps that are necessary to detect the proteins of interest. As a result, the proteins in the gel are transferred onto a membrane made of nitrocellulose or PVDF by applying a current. (See the Secondary Probing section below for more information about the difference between nitrocellulose and PVDF.) This is the actual blotting process and is necessary in order to expose the proteins on a thin surface layer to the probe (see below). The membrane has protein binding properties and binds proteins non-specifically (i.e. binds all proteins equally well). Protein binding is based upon hydrophobic interactions, as well as charged interactions between the membrane and protein.

3) **Blocking**: The membrane is then blocked, in order to prevent non-specific protein interactions between the membrane and the antibody protein (next step, below). This is done by placing the membrane in a solution of BSA, non-fat dry milk, and detergents such as Tween 20 or colloidal carbon. Without the blocking, the probe would bind non-specifically to the membrane.

4) **Probing**: The location of the antibody is revealed by incubating it with a colorless substrate that the attached enzyme converts to a colored product that can be seen and photographed.

   * **Primary Antibody**

   An antibody (often called the primary antibody), which ideally recognizes only the protein of interest, is incubated with the membrane. The antibody is diluted in a solution containing a modest amount of a salt such as sodium chloride, some protein (such as BSA) to prevent non-specific binding of the antibody to surfaces, and a small amount of a buffer to keep the solution near neutral pH. The diluted antibody solution and the membrane can be sealed in a plastic bag and gently agitated for an "incubation" of about half an hour. The primary antibody should not bind any of the other proteins on the membrane; it is obtained by immunizing an animal (usually a rabbit or goat) with the protein of interest (i.e., injecting the protein into the animal's body), and then collecting the antibodies the animal produces against that protein. Some high affinity monoclonal antibodies can also be used for Western blots.

   * **Secondary Antibody**
After rinsing the membrane to remove unbound primary antibody, a secondary antibody is incubated with the membrane. This antibody binds to the first antibody, and is usually produced by a different animal. (For example, goat anti-rabbit antibody might be used if the first antibody was produced by rabbits.) This secondary antibody is usually linked to an enzyme that can allow for visual identification of where on the membrane it has bound. As with the ELISPOT and ELISA procedures, the enzyme can be provided with a substrate molecule that will be converted by the enzyme to a colored reaction product that will be visible on the membrane (see the figure below with blue bands). Alternately, the reaction product may produce enough fluorescence to expose a sensitive sheet of film when it is placed against the membrane. A third alternative is to use a radioactive label rather than an enzyme coupled to the secondary antibody, such as labeling an antibody-binding protein like Staphylococcus Protein A with a radioactive isotope of iodine.

Since the first antibody only recognizes the protein of interest, and the second antibody only recognizes the first antibody, if there is stain present on the membrane then the protein of interest must also be present on the membrane. Thus, the protein bands on the membrane that are stained contain the protein that was to be detected, the other locations on the membrane do not. Size approximations can be done by comparing the stained bands to that of a pre-stained protein size marker.

5) Detection: After the unbound probes are washed away, the Western blot is ready for detection of the probes that are labeled and bound to the protein of interest. Several different methods of detection exist.

Fluorescent detection: A fluorescently labeled probe is excited by light and the emission of the excitation is then detected by a photosensor such as CCD camera equipped with appropriate emission filters that capture a digital image of the western blot and allows further data analysis such as molecular weight analysis and a quantitative western blot analysis. Fluorescence is considered to be among the most sensitive detection methods for blotting analysis.
Picture of a western blot with 9 vertical lanes. The first lane (M) corresponds to the molecular weight marker. Lanes 1 though 8 correspond to the protein samples.
### APPENDIX D

**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM</td>
<td>atomic force microscopy/microscope</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CMOS</td>
<td>complementary metal oxide semiconductor</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammogram</td>
</tr>
<tr>
<td>DIP</td>
<td>dual-in-line pin</td>
</tr>
<tr>
<td>Eco</td>
<td>synthesized by constant potential</td>
</tr>
<tr>
<td>Ecy</td>
<td>3,4-ethylene dioxythiphene</td>
</tr>
<tr>
<td>EP</td>
<td>electronic polymer</td>
</tr>
<tr>
<td>E-SEM</td>
<td>environmental scanning electron microscope</td>
</tr>
<tr>
<td>Fc&lt;sup&gt;+&lt;/sup&gt;/Fc</td>
<td>ferrocenium/ferrocene couple</td>
</tr>
<tr>
<td>FE-SEM</td>
<td>field-emission scanning electron microscope</td>
</tr>
<tr>
<td>FET</td>
<td>field effect transistors</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>IDA</td>
<td>interdigitated arrays</td>
</tr>
<tr>
<td>I-V</td>
<td>current-voltage</td>
</tr>
<tr>
<td>M</td>
<td>molarity</td>
</tr>
<tr>
<td>NHE</td>
<td>normal hydrogen electrode</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PEDOT</td>
<td>poly(3,4-ethylene dioxythiphene)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Ppy</td>
<td>poly(pyrrole)</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>PSS</td>
<td>poly(styrene sulfonic acid)</td>
</tr>
<tr>
<td>PSU</td>
<td>Pennsylvania State University</td>
</tr>
<tr>
<td>S</td>
<td>siems</td>
</tr>
<tr>
<td>SCE</td>
<td>saturated calomel electrode</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>NHS-fluorescein</td>
<td>5-(and 6)-carboxyfluorescein, succinimidyl ester</td>
</tr>
<tr>
<td>sulfo-NHS-LC-LC-biotin</td>
<td>sulfosuccinimidyl-6-(biotinamido)-6-hexanamido hexanoate</td>
</tr>
<tr>
<td>TBAPF$_6$</td>
<td>tetrabutyl ammonium hexafluorophosphate</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscope</td>
</tr>
<tr>
<td>UV-VIS</td>
<td>ultra violet-visible spectroscopy</td>
</tr>
<tr>
<td>V</td>
<td>volts</td>
</tr>
<tr>
<td>Vrms</td>
<td>root mean square voltage</td>
</tr>
<tr>
<td>v/v</td>
<td>volume of solute/total volume</td>
</tr>
<tr>
<td>v/w</td>
<td>volume of solute/total weight</td>
</tr>
<tr>
<td>Ω</td>
<td>ohm</td>
</tr>
</tbody>
</table>
Vita

Rose M. Hernández

Rose M. Hernández was born in San Juan, Puerto Rico; the daughter of Efraín Hernández and María L. Reyes. She grew up in one of the public housing projects of the city of San Juan, where she grew comfortable with her surrounding but deep inside it was not to be her future. She attended the University of Puerto Rico’s high school thanks to her father’s efforts to take her out of the public school system. Having witness her mother’s love for nursing, she develop a desire for medicine that lead her to initiate her career in chemistry. After graduating from high school she attended the beautiful campus of the University of Puerto Rico at Cayey (UPR-Cayey), where she earned her Honors College B.S. degree magna cum laude in 1997.

The many wonderful professors at UPR-Cayey encouraged her to pursue a Ph.D. in science. After graduating, she worked at the National Institute of Standards and Technology (NIST) in Gaithersburg, MD, and a year later she began graduate studies in Materials Science at The Pennsylvania State University (PSU). In the spring of 2001, she joined Dr. Thomas E. Mallouk’s research group while working at NIST, and in the fall of 2003 she relocated to PSU to continue her research studies. She earned her Ph.D. in December 2005.

The distance from her roots taught Rose great things about herself and life. She discovered that she is full of passion, and that passion is what fuels her life. She loves dancing, and she appeared in several Latin show-cases in various cities. She also realized that there is a whole world to explore, and that the way back home may take a little longer. The same distance taught her about family, friendship, and love, and in many wonderful ways brought her closer to God.