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Higher Energy Gap Control of Fluorescence in Conjugated Polymers

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In memory of

Kao, Liao Pi-Shuang

1929-2017
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# ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
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<th>Full Form</th>
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<tbody>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
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<tr>
<td>CP</td>
<td>Conjugated Polymer</td>
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<tr>
<td>CV</td>
<td>Cyclic Voltammogram</td>
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<tr>
<td>Cys</td>
<td>Cysteine</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
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<tr>
<td>DIPA</td>
<td>Diisopropylamine</td>
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<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>ET</td>
<td>Energy Transfer</td>
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<tr>
<td>FRET</td>
<td>Fluorescence Resonance Energy Transfer</td>
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<td>F</td>
<td>Fluorescence Intensity</td>
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<tr>
<td>GPC</td>
<td>Gel-permeation Chromatography</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
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<tr>
<td>Hey</td>
<td>Homocysteine</td>
</tr>
<tr>
<td>HEPES</td>
<td>(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
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<tr>
<td>ICT</td>
<td>Intramolecular Charge Transfer</td>
</tr>
<tr>
<td>ITO</td>
<td>Indium-tin Oxide</td>
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<tr>
<td>PET</td>
<td>Photoinduced Electron Transfer</td>
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<tr>
<td>LUMO</td>
<td>Lowest Unoccupied Molecular Orbital</td>
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<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>OFET</td>
<td>Organic Field Effect Transistor</td>
</tr>
<tr>
<td>OLED</td>
<td>Organic Light-emitting Diode</td>
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<tr>
<td>OPV</td>
<td>Organic Photovoltaic</td>
</tr>
<tr>
<td>OPE</td>
<td>Oligo(phenylene ethynylene)</td>
</tr>
<tr>
<td>P3HT</td>
<td>Poly(3-hexylthiophene)</td>
</tr>
<tr>
<td>PAc</td>
<td>Polyacetylene</td>
</tr>
<tr>
<td>PAE</td>
<td>Poly(arylene ethynylene)</td>
</tr>
<tr>
<td>PANI</td>
<td>Polyaniline</td>
</tr>
<tr>
<td>PAV</td>
<td>Poly(arylene vinylene)</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-Buffered Silane</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity Index</td>
</tr>
<tr>
<td>PEDOT</td>
<td>Poly(3,4-ethylenedioxythiophene)</td>
</tr>
<tr>
<td>PFO</td>
<td>Polyfluorene</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methyl-methacrylate)</td>
</tr>
<tr>
<td>PPE</td>
<td>Poly(phenylene ethynylene)</td>
</tr>
<tr>
<td>PPETE</td>
<td>Poly[para-(phenylene ethynylene)-alt-(thienylene ethynylene)]</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts-per-million</td>
</tr>
<tr>
<td>PPP</td>
<td>Poly(phenylene)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>PPV</td>
<td>Poly(\textit{para}-phenylene vinylene)</td>
</tr>
<tr>
<td>PPy</td>
<td>Polypyrrole</td>
</tr>
<tr>
<td>PT</td>
<td>Polythiophene</td>
</tr>
<tr>
<td>PTV</td>
<td>Poly(thiénylene vinylene)</td>
</tr>
<tr>
<td>RMS</td>
<td>Root Mean Square</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium Fluoride (Bu$_4$NF)</td>
</tr>
<tr>
<td>TEG</td>
<td>Triethylene Glycol</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>tmeda</td>
<td>$N,N,N'$-trimethylethlenediamine</td>
</tr>
<tr>
<td>UV-vis</td>
<td>Ultraviolet-visible</td>
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ABSTRACT

Chemo- and biosensors based on fluorescent conjugated polymer benefit from greater detection sensitivity due to amplification of the electronic perturbations produced by analyte binding. This amplification stems from the exciton-transporting properties of conjugated polymers. A conventional design paradigm relies on the analyte binding events which generate sites of lower energy relative to the polymer energy: either fluorescence quenching sites (turn-off sensors) or bathochromically shifted fluorophores (turn-on sensors). In both type sensors, the excitons migrate to the lower-energy site created by analyte binding.

This dissertation primarily focused the investigation of an alternative paradigm when analyte binding creates higher energy gap sites in the polymer backbone. Such higher-energy gap sites act as “roadblocks” for excitons to reduce their migration length. Decreasing exciton migration length is accompanied by increasing fluorescence intensity, thus generating an amplified turn-on fluorescent response. The new paradigm expands the generality and universality of the signal amplification concept in conjugated polymers, and can be used to design amplifying turn-on fluorescent sensors for various practically useful analytes such as hydrogen sulfide and cysteine.

In the last part of this dissertation, we present a series of poly(p-phenylene ethynylene) thin films prepared by stepwise surface-initiated polymerization. In addition to experimental simplicity and reproducibility of the preparation, and broad variety of compatible building blocks, this method requires low material consumption and no purification for the final bulk thin films. The stepwise surface-initiated polymerization yields dense films of covalently immobilized polymer chains with precisely controlled molecular structure and organization.
CHAPTER 1. INTRODUCTION TO CONJUGATED POLYMERS AS CHEMOSENSORY MATERIALS

1.1 Overview of Conjugated Polymers

Conjugated polymers (CPs) are organic macromolecules with $\pi$-conjugation delocalized over a large number of repeating units.\(^1\) The field of conjugated polymers became popular since finding of metallic electrical conductivity in oxidatively doped polyacetylene (PAc) films by Shirakawa, Heeger, and MacDiarmid in 1977 (in 2000 they received Nobel Prize in Chemistry for that discovery).\(^2\)–\(^4\) They found that the conductivity of polyacetylene increased nearly ten orders of magnitude upon exposure of the polymer film to iodine.\(^5,6\) Since that initial studies, extensive research on the photophysical, electrochemical, and magnetic properties of conjugated polymers has been a popular target in academic community.\(^7\) In addition to fundamental research significance, the tunable electronic and optical properties, flexibility, and solution processability made conjugated polymers a promising class of materials for the next generation electronics such as organic light-emitting diodes (OLEDs),\(^8\) organic field-effect transistors (OFETs),\(^9\) photorefractive devices,\(^10\) organic photovoltaic cells (OPVs),\(^11\) and chemosensory materials.\(^12\)–\(^16\)

![Figure 1.1. Representative chemical structures and abbreviated names of common conjugated polymers.](image)

These properties mainly stem from (1) the extended $\pi$-electron delocalization over entire conjugated polymer backbone which reduces the energy gap between the valence and the conduction bands down to 1.5–3.0 eV; and (2) the efficiency of inter- and intramolecular excitation
energy (exciton) migration, especially in the condensed and solid state. Figure 1.1 shows common conjugated polymers such as poly(\textit{para}-phenylene) (PPP), poly(\textit{para}-phenylene vinylene)s(PPV), poly(\textit{para}-phenylene ethynylene) (PPE), polythiophene (PT), poly(thienylene vinylene) (PTVs), poly(3,4-ethylenedioxythiophene) (PEDOT), polyaniline (PANI), polypyrrole (PPy), and polyfluorene (PFO).\textsuperscript{17}

1.2 Inter- and Intramolecular Excitation Energy Transfer

It is important to understand how the electronic excitation energy (exciton) transfers in/between conjugated polymer chains prior to designing OLED, OFET, solar cells, and chemosensory devices.\textsuperscript{18} A quasi-particle exciton is generally referred to the excitation energy and the surrounding environment changes in bond length and angles along the $\pi$-conjugated system in response to the formation of the excited state.\textsuperscript{19}

![Schematic diagrams showing mechanisms of Förster (top) and Dexter (bottom) energy transfer processes.](image)

Generally, excitation energy can migrate intermolecularly and/or intramolecularly, in which the former occurs via dipole–induced dipole through-space interactions (Förster-type)\textsuperscript{20} and the latter happens by the combination of through-space mechanism and via the through-bond mechanism occurring through strong orbital overlap between chromophores (Dexter-type) (Figure
Nevertheless, whether the Förster-type or Dexter-type mechanism dominates the excitation energy transfer in conjugated polymers still remains a point of debates in research literature owing to the difficulty of quantitative differentiating one from the other. In an ideal scenario of strong intrachain electronic coupling where through-bond Dexter-type mechanism dominates, excitons one-dimensionally walk along the isolated polymer chains whose structure is hypothesized to be a defect-free planar conformation with complete electron delocalization along the backbone. In reality, however, the disordered nature of conjugated polymers tends to form abundance of structural defects such as kinking, coiling, twisting, etc., whereby these inherently defects can greatly inhibit Dexter energy migration since the orbital coherence between segmented chromophores is discontinuous (Figure 1.3).

Figure 1.3. Example of single-chain conformation of a poly(phenylene vinlyene) conjugated polymer, referred to as the defect cylinder conformation. Conformational disorder produces a chain of linked chromophores (or conformational subunits) outlined conceptually by the boxes. The intramolecular excitation energy transfer (migration along the backbone) is the predominant mechanism when the polymer chain assumes an open, extended conformation, typical for solutions in good solvents such as chloroform; on the other hand, intermolecular interactions (hopping between segments in close proximity) are dominant for tightly coiled configurations, typically found in polymer nanoparticles, or thin films. Reproduced with permission from Ref. 24. Copyright © 2009 The American Association for the Advancement of Science.
The luminescence signature of intermolecular migration usually combines bathochromic emission, longer lifetime, and lower quantum yield, since delocalization between segments reduces the excited state energy relative to the isolated chain exciton. A detailed elucidation of these two mechanisms was presented by Schwartz et al.\textsuperscript{25} In that work, isolated extended alkoxy-PPV macromolecular chains were incorporated into long cylindrical pores of an extended silica matrix in order to eliminate any intermolecular interactions, and the polarized luminescence spectroscopy showed that the excitation energy diffusion along the isolated polymer backbone was a slower process ($10^{-11} \sim 10^{-10}$ s) due to weak dipole coupling along the extended polymer chain. At the same time, intermolecular energy migration was found to be much faster ($10^{-12} \sim 10^{-11}$ s).

Although intramolecular energy transfer is generally considered less efficient than its intermolecular counterpart, some recent studies have shown that the intramolecular migration in isolated polymers may be actually efficient. Swager et al. found that intramolecular energy transfer indeed was the major pathway for excitation migration in conjugated polymers in anisotropic Langmuir-Blodgett films of monolayered PPE.\textsuperscript{26–28} The substantial contribution of through-bond Dexter-type mechanism of excitation energy transfer along the conjugated polymer backbone was found in chain-extended conformations of uniformly aligned PPEs in nematic liquid crystalline media (Figure 1.4).\textsuperscript{29} Unlike through-space hopping by Förster-type route, excitation energy transfer by orbital overlap is dramatically affected by structural defects and molecular alignments. This significance was elegantly addressed by dissolving PPE derivatives in nematic liquid crystals, allowing finely manipulation of the chain-extension lengths and molecular alignments of the PPE backbone. The end-capped low-energy gap fluorophores was introduced to accept intramolecular excitations and therefore the efficiency of energy migration could be evaluated via site-selective emission of the termini. When the temperature was increased above the nematic-isotropic
transition of the liquid crystal, the dramatically diminished fluorescence from the terminal fluorophores, along with simultaneous increase in the backbone PPE emission strongly indicated the disrupting of conjugated length and related diminishing of the energy migration by Dexter-type mechanism.

Figure 1.4. Simplified representation of conjugated polymer PPE in isotropic and liquid crystalline solutions. In isotropic solution, the conformational disorder in the polymer backbone prevents efficient intramolecular exciton migration, thus resulting in predominant emission from the PPE backbone. In nematic liquid crystalline solution, the increased electronic conjugation in the straightened and planarized polymer chains is higher, which leads to the enhanced intramolecular migration toward the terminal groups with a concomitant increase in the termini’s emission. Reproduced with permission from Ref. 29. Copyright © 2005 American Chemical Society.

Quantitative differentiation of the contribution from intermolecular and intramolecular energy transfer pathways can be performed by subensemble analytical methods such as cryogenic single molecular spectroscopy. This analytical method is based on the measuring zero-phonon transition line accompanying distinct vibrionic band of individual chromophores within a polymer chain, and thereby can differentiate between intermolecular, intramolecular, and dynamic modes of disorder. Lupton et al. recently investigated poly(3-hexylthiophene) (P3HT) in Zeonex480 and poly(methyl methacrylate) (PMMA) matrices, to simulate isolated and aggregated polymer chains, respectively. They found that single chromophore in the conjugated chain was flexible and could adopt wide range of subtly varying conformations (e.g. bending of backbone, chain torsion, and
changes in bond alternation) that affect the adjacent chromophore in a single chain through coherent oscillation, which is also responsible for intermolecular energy transfer.

Förster and Dexter mechanisms both contribute greatly to energy transfer in conjugated polymers. Although the Förster mechanism is considered to play a major role in high concentration solutions and aggregates states due to close interchain distance, Dexter energy transfer, which is based on orbital overlap along $\pi$-electron delocalized conjugated backbone, is efficient in more dilute solutions.

1.3 Signal Amplification in Conjugated Polymer Sensors

A photon absorption generates a strongly bound electron-hole pair (exciton) that can randomly walk along the conjugated system as well as hop from one conjugated system to another before a photophysical and/or photochemical process occurs. By using site-selective fluorescence technique, it was found that inherent Stokes shift in conjugated polymers is smaller than that induced by exciton migration. Therefore, the photoluminescence occurring from the lower energy gap segments gives conjugated polymer a larger Stokes shift and inhomogeneous featureless luminescence spectrum. In case of an isolated polymer chain, excitation energy migration happens intramolecularly in a slow process relying on orbital overlap,\textsuperscript{31} and the diffusion length of exciton ranges from 5 to 14 nm.\textsuperscript{32,33} Swager \textit{et al.} demonstrated, by end-capping poly(\textit{para}-phenylene ethynylene)s with a lower energy gap anthracene end groups, that the exciton effectively migrates to the lowest energy gap site (Figure 1.5).\textsuperscript{34}

On the other hand, conjugated polymer photoluminescence can be efficiently quenched via a photoinduced electron transfer (PET) process, where excited state electron on the conduction band is brought back to the valence band by passing through the LUMO of an electron-deficient analyte bound to the polymer backbone. Combined with an efficient exciton migration (especially
when it becomes a three-dimensional process in solid and condensed states), this process is known as “turn-off” amplification mechanism. Swager et al. and Whitten et al. independently demonstrated that electron-deficient molecules such as nitro-aromatic explosives can effectively bind to electron-reach conjugated polymers (Figure 1.6).\textsuperscript{35,36,37} They showed that a single chemical binding or unbinding event can trigger electronic perturbations along the entire \( \pi \)-electron conjugated backbone, thereby the collective response can be measured optically or electrochemically. It is important to note that such a response is dominated by static quenching.

![Figure 1.5](image)

**Figure 1.5.** Representation of energy migration in a conjugated polymer molecular wire with a decrease in band gap at the termini. Reproduced with permission from Ref. 34. Copyright © 1995 American Chemical Society.

In contrast to the “turn-off” mechanism where the metal-ligand coordination or van der Waals interactions between the polymers and the electron-deficient quenchers are responsible for the photoluminescence quenching, Kim and Swager demonstrated an example of an unprecedented “turn-on” fluorescence amplifying conjugated polymer for fluoride anion detection.\textsuperscript{38} In Figure 1.7, a non-emissive pendant fluoride-reactive group, electronically coupled with the conjugated polymer backbone, lactonizes to form a lower-energy coumarin fluorophore after treating with analytical amount of fluoride anion. This forces the excitons produced in the polymer backbone to migrate to the lower-energy coumarin site with accompanying increase in the coumarin fluorescence. The resulting fluorescence amplification is approximately 100 times with respect to
a small molecule analog. However, there are only a limited number of suitable analytical reactions that can form a lower-energy highly fluorescent fluorophore where its absorption and emission are bathochromically shifted relative to the CP backbone. This indeed intrinsically limits the generality and application potential of this approach.

Figure 1.6. Conceptual illustration of energy migration to a receptor site occupied by PET quencher methyl viologen (paraquat, PQ$^{2+}$). When the excited electron encounters a receptor site with a bound PQ$^{2+}$ group, electron transfer quenching occurs. Reproduced with permission from Ref. 37. Copyright © 1995 American Chemical Society.

Figure 1.7. Schematic band diagram illustrating the mechanism by which a semiconducting polymer can produce an enhanced *turn-on* fluorescence chemosensory response. The horizontal dimension represents the position along the polymer backbone shown schematically at the bottom. Excitons are created by absorption of a photon ($h\nu$) and they migrate along the polymer backbone. Fluoride-induced lactonization of the electronically coupled receptor groups produces an exciton trapping site with a smaller band gap ($E_g$) and recombination of excitons at that site results in a new amplified emission. Reproduced with permission from Ref. 38. Copyright © 2003 Wiley-VCH.

To address this challenge, Nesterov et al. introduced an alternative “higher energy gap” paradigm — where instead of relying on the formation of a smaller energy gap emissive site where
excitation energy from the conjugated polymer backbone is funneled, the generation of a local higher energy gap site in the π-conjugated backbone is able to restrict the energy migration along the isolated π-electron conjugated system. Importantly, this concept relies on the small changes at the analyte-reactive site rather than on drastic changes in the π-electron conjugated backbone. Poly(p-arylene vinylene)s (PAVs) emerged as an excellent conjugated polymer choice for this mechanism due to its extraordinary emissive nature and low energy band gap ($\lambda_{em} > 500$ nm), as well as a better feasibility to functionalize with analyte-specific receptor groups compared to other classes of conjugated polymers. In the initial study, diethyl chlorophosphate (DCP), a commonly used mimics of organophosphorus warfare agents, was chosen as an analytical detection target. The PAV polymer sensor incorporated naphthalene based hydroxy oxime reactive group that can facilely convert to the corresponding isoxazole upon reacting with DCP. The formation of isoxazole unit, which possesses a noticeable higher HOMO-LUMO gap relative to the unreacted hydroxy oxime, resulted in an amplified turn-on fluorescent response. Since the turn-on response in the higher energy gap mechanism is based on modulation of the intramolecular exciton migration in CPs, it produces an amplification effect similar to that observed in cases of turn-off sensors for electron deficient analytes.

Figure 1.8. Schematic diagram illustrating the higher energy gap control of CP fluorescence: an analyte (red circle) binding creates a local higher energy gap site in the CP backbone which acts as a “roadblock” and decreases the length of the excitation/exciton migration along the π-conjugated system, resulting in the increase of fluorescent intensity of the conjugated polymer. Reproduced with permission from Ref. 39. Copyright © 2013 American Chemical Society.
1.4 Turn-On Sensory Systems Based on Fluorescent Conjugated Polymers

1.4.1 Sensing Metal Ions

In contrast to turn-off, quenching-based conjugated polymer fluorescent sensors, research literature describes rather limited number of turn-on conjugated polymer based fluorescent sensors. This is probably related to the difficulties with general design principles of such sensors. Below, we provide an overview of known turn-on sensors based on conjugated polymers. Many of such sensors are based on binding metal cations by chelating ligands which results in diminishing PET quenching process. Jones et al. reported sensor 1 based on poly[p-(phenylene ethylene)-alt-(thienylene ethynylene)] containing N,N,N’-trimethylethylenediamine (tmeda-PPETE) pendant units that showed turn-on selective fluorescent response on Hg$^{2+}$ over Ca$^{2+}$, Zn$^{2+}$ and H$^+$ at the submicromolar concentrations.$^{40}$ The low quantum yield of the pristine polymer ($\phi_{FL} = 0.09$) was attributed to PET from the conjugated backbone to the amino group of the uncomplexed receptor tmeda. Upon binding with Hg$^{2+}$, a stable tmeda/Hg$^{2+}$ complex was formed which lowered the HOMO of the receptor and inhibited the PET process, resulting in the increase of the fluorescence intensity. Fan and Jones also prepared an inorganic/organic hybrid complex, tmeda-PPETE/Cu$^{2+}$, which showed selective response on detection of Fe$^{2+}$ cations.$^{41}$ In the initial state, the pre-coordinating with Cu$^{2+}$ completely quenched the polymer fluorescence.

Upon the addition of Fe$^{2+}$, tmeda-PPETE/Cu$^{2+}$ hybrid system showed a gradual enhancement in fluorescence up to 150-fold, and was highly selective toward Fe$^{2+}$ over Ca$^{2+}$, Hg$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Mn$^{2+}$, and H$^+$ ions. The authors proposed that the amplified fluorescent response not only simply resulted from the difference in association constants between tmeda and various metal ions, but was due to the competitive binding between Cu$^{2+}$ and other metal ions.
Zhu and co-workers reported a poly($p$-phenylene ethynylene) derivative 2 incorporating a single diastereomer ($R,R$)-salen unit as a Zn$^{2+}$ receptor that showed a 7.8-fold increase in fluorescence upon gradual addition of Zn$^{2+}$. The formation of Zn$^{2+}$ metallopolymer significantly suppressed PET quenching process as well as resulted in planarization of the polymer backbone to increase the $\pi$-electron conjugation. Noteworthy, 2 exhibited a good selectivity for Zn$^{2+}$ over Cd$^{2+}$ and Hg$^{2+}$ despite all three ions being in the same group of the periodic table and having closed-shell configurations.

Li and co-workers also investigated Hg$^{2+}$ detection by the rhodamine incorporating poly[$p$-(phenylene ethylene)-alt-(thienylene ethynylene)] sensor polymer 3. Changes that were observed upon addition of Hg$^{2+}$ in both absorption and fluorescence spectra could be attributed to FRET from the conjugated backbone to the ring-opened isomer of rhodamine. It was found that the fluorescence intensity ratio of conjugated backbone to rhodamine emission bands increased by a factor of 18.6. Sensor 3 showed good selectivity toward Hg$^{2+}$, even over the background of 20 equiv. of metal ions including Li$^+$, Na$^+$, K$^+$, Ca$^{2+}$, Ba$^{2+}$, Mg$^{2+}$, Al$^{3+}$, Ag$^+$, Cu$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$.

Recently, Hua and co-workers have prepared poly(fluorene-co-thiocarbonyl quinacridone) Hg$^{2+}$ fluorescence turn-on probe 4, which demonstrated excellent sensitivity and 32-fold fluorescent intensity increase upon analyte binding. The fluorescent emission of 4 was initially
suppressed by the presence of thiocarbonyl moiety in the conjugated backbone. Addition of mercury ion resulted in conversion of thiocarbonyl to carbonyl functional group bringing about the absorbance change and turn-on intensity increase in fluorescence. Furthermore, Hua also demonstrated that the nanoparticles of 4 expressed good sensitivity and selectivity toward Hg$^{2+}$ in PBS solution and in the presence of HeLa cells.

Wang et al. reported a K$^+$ sensor based on excitation energy transfer from water-soluble polyfluorene 5 to the fluorescein labelled G-rich single-stranded DNA. With quaternary
ammonium cations in the side chains, 5 can electrostatically attract to the negatively charged G-rich ssDNA and form a 1:1 complex with it. The loose complex of 5 and random coiled ssDNA showed a predominant fluorescence band from 5 at 422 nm and a low-intensity band at 527 nm due to an inefficient FRET from polymer 5 to fluorescein chromophore. Addition of the complex of 5 and fluorescein-labeled ssDNA to an aqueous solution of K\(^+\) resulted in the significant turn-on increase of the fluorescein fluorescence band, which was due to shortening the distance between 5 and G-quadruplex forming in the presence of K\(^+\). The FRET ratio \((I_{527}/I_{422})\) of the polyfluorene to fluorescein emission bands increased about 2, 3.4, and 16 times in the presence of 8.5, 30, and 50 mM KCl, respectively. Selectivity examination showed a much weaker response on the addition of Mg\(^{2+}\) and Ca\(^{2+}\); and negligible response on Na\(^+\), NH\(_4\)\(^+\), and Li\(^+\) due to the inability of these ions to facilitate formation of the G-quadruplex secondary structure.

Smith et al. exploited benzo-15-crown-5-ether based poly(\(p\)-phenylene vinylene)s derivative (CE-OPV) 6 as an Eu\(^{3+}\) chemosensor. Sensor 6 represented a rare case when a crown-ether based conjugated polymer exhibited turn-on fluorescence enhancement since ion-enhanced aggregation typically effectively traps and non-radiatively dissipates the emissive energy. The authors argued that the fluorescence enhancement could be caused by suppression of ICT.

Zhu et al. have prepared a chiral conjugated polymer by joining a \((R,R)\)-salen ligand with a perylenetetracarboxylic acid derivative. Conjugated polymer sensor 7 exhibited only a weak
fluorescent band at 635 nm prior to coordinating with added Hg\textsuperscript{2+}, and the authors proposed that it was attributed to an efficient intramolecular charge transfer (ICT) between the salen and perylenetetracarboxylate units along the polymer backbone. They observed a significant (up to 26 times) increase of fluorescence intensity upon addition of Hg\textsuperscript{2+} with an excellent selectivity.

1.4.2 Small Organic Molecules and Bioanalytes

Lippard and co-workers reported a bipyridyl-incorporating poly(p-phenylene vinylene) conjugated polymer sensor 8 to detect NO gaseous molecule with nanomolar sensitivity.\textsuperscript{49} \(8/\text{Cu}^{2+}\) complex showed a moderate fluorescence quenching, whereas the introduction of 300 eq. of NO increased the polymer emission by 2.8-fold. The authors proposed that in alcohol solution, the NO can reduce paramagnetic Cu\textsuperscript{2+} cation to diamagnetic Cu\textsuperscript{1+} cation; the paramagnetic properties of Cu\textsuperscript{2+} were mainly responsible for the initial fluorescence quenching.

Li and co-workers prepared a polyfluorene derivative 9 containing imidazole pendant side groups that are chelating toward Cu\textsuperscript{2+} to be used as a cyanide turn-on sensor.\textsuperscript{50} Excitation energy transfer from the conjugated polymer backbone to imidazole-bound Cu\textsuperscript{2+} lead to dramatic reduction in fluorescence emission at 402 nm while the replacement of imidazole with other
nitrogen-containing heterocyclic units would not be sufficient to diminish the emission. 9/Cu$^{2+}$ complex showed a significant fluorescence response upon the addition of CN$^-$. 

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\text{Zhu et al. demonstrated that a chiral imine-based conjugated polymer 10 incorporating both \((S\)-2,2$'$-binaphthol (BINOL) and \((S\)-2,2$'$-binaphthyldiamine (BINAM) units can selectively recognize \((D\)-phenylalaninol over its enantiomer \((L\)-phenylalaninol.$^{51}$ The binding of \((D\)-phenylalaninol to the chiral polymer backbone inhibited PET process and therefore triggered the fluorescence turn-on response. The enantioselective response of 10 can be attributed to the formation of a more stable 10/(\(D\)-phenylalaninol diastereomer complex stemming from the interactions of hydroxy and imine functional groups on BINOL and a target amino alcohol. Noteworthy, reduction of imine units upon the treatment with NaBH$_4$ converted 10 in a non-conjugated polymer which showed a much lower fluorescence response. The polymer 10 showed an enantiomeric fluorescence difference ratio ($\epsilon_f$) of 6.85-fold for \((D\)-phenylalaninol with respect to its enantiomer. The authors unveiled good analyte selectivity of 10 since no significant fluorescence enhancement was noticed upon addition of \((D)/(L\)-mandelic acid, \((R)/(S)-
phenylethylamine, and \((D)/(L)\)-phenylglycinol. The authors further extended the concept of enantioselective recognition for the sensing chiral \((L)\)-\(\alpha\)-hydroxycarboxylic acids.\(^{52}\) \((R,R)\)-salen incorporating poly(p-phenylene ethynylene) 11 showed only a weak fluorescence since the strong intramolecular hydrogen bonding between the hydroxy and imine groups on the salen moiety quenched the fluorescence of 11, whereas the fluorescent emission could be subsequently restored by binding of target \((L)\)-\(\alpha\)-hydroxycarboxylic acids. The sensor 11 showed \(ef\) of 8.41 for \((L)\)-mandelic acid over \((D)\)-mandelic acid, and 6.55 for \((L)\)-lactic acid over \((D)\)-lactic acid. Reduction of the imine group to saturated amine resulted in a disordered and flexible polymer with a strong intrinsic fluorescence. In contrast to polymer sensor 11, the reduced disordered polymer showed formation of an unstable complex with mandelic acid which was acting as a site for nonradiative relaxation, leading to fluorescence quenching (turn-off response).

Recently, Pu and co-workers reported a chiral 1,1'-bis-2-naphthol based conjugated polymer 12 whose fluorescence was initially quenched by the aldehyde groups.\(^{53}\) Treating the enantiomeric polymer \((S)\)-12/Zn\(^{2+}\) complex with \((R)\)-leucinol yielded a large increase in fluorescence emission at 535 nm, whereas a weaker fluorescence increase was observed for \((L)\)-leucinol. In addition, good selectivity over various 2-aminoalcohols was observed. The authors hypothesized that the most sterically bulky substituent adjacent to the chiral amine center gave the highest \(ef\) value. The importance of having conjugated polymer entity was demonstrated by the
fact that a small molecule counterpart of the polymer 12 showed only a negligible difference in the enantioselective fluorescent response.

Wang et al. designed conjugated hydroxy-functionalized polyquinoline polymer 13 as a fluoride anion sensor, which operated based on the formation of hydrogen bonding between $F^-$ and the hydroxy group. Addition of $F^-$ resulted in the appearance of a new absorption peak at around 500 nm, as well as significantly increased the fluorescence band at 620 nm up to 147-fold. In contrast, a small molecule counterpart of the polymer 13 exhibited only a much smaller 14.7 times fluorescent enhancement. The authors proposed that the turn-on sensing effect involved two mechanisms: the formation of phenolate induced by $F^-$ and the ICT between quinolone and the phenolate moieties. The former was investigated by $^1$H NMR using a small molecule analog which showed diminishing of the hydroxy peak. The role of the second mechanism was clearly indicated by the long range bathochromic shift in absorption and emission spectra. Additionally, no obvious change occurred upon addition of $Cl^-$, $Br^-$, and $H_2PO_4^-$.  

Thomas and Swager reported an amino-functionalized poly($p$-phenylene) 14 whose fluorescence could be increased by adding trace amounts of a reducing agent hydrazine. Although 14 showed such an enhancement only in thin films, the observed hydrazine detection
limit was 100 ppb. It was likely due to the presence of inherently oxidized defect sites in the bulk materials as well as the much greater exciton migration in conjugated polymers in solid state relative to an isolated chain in dilute solution. It is noteworthy that less electron-rich 15 and 16 showed lower response compared to 14, indicating that most readily oxidized polymer gave the largest fluorescence enhancement.

Tian et al. prepared naphthalimide-functionalized poly(phenylacetylene) 17 which exhibited both fluorescent ratiometric and colorimetric responses on addition of F⁻. In the presence of F⁻, ICT could be facilitated by the conversion of amide (electron donor) to imine (electron acceptor) moiety, resulting in a large electron density redistribution and showing charge transfer peaks in the UV-vis and emission spectra. 17 demonstrated good selectivity for fluoride over other halogen anions such as Br⁻, Cl⁻, and I⁻.

Kakuchi and co-workers introduced a concept of anion/urea halogen bonding promoted deaggregation that unquenched the fluorescence of conjugated polymers. Urea-functionalized poly(phenylenebutadiynylene) 18 intrinsically favors interchain aggregation since the abundant urea units tend to form multiple intermolecular hydrogen bonds, which resulted in the aggregation-induced quenched polymer emission band at 460 nm. Upon addition of F⁻, the stronger association between F⁻ anion and urea resulted in disassembly of the aggregated state of 18 thereby producing
a new fluorescence band at 493 nm originating from the isolated polymer. Polymer sensor 18 showed a good response on F⁻; however, it also showed a relatively low selectivity and lacked discrimination against other anions such as AcO⁻, BzO⁻, and N₃⁻.

In a sharp contrast to the above-described work, Zhao and Schanze developed a conjugated polymer sensor where anion-enhanced aggregation resulted in a turn-on ratiometric fluorescent response.⁵⁹ The cationic polyelectrolyte poly(p-phenylene ethynylene) 19 bearing ammonium-functionalized side chains showed excellent water solubility and intense blue fluorescence at 432 nm with well resolved vibronic structure. The intensity of the emission band at 432 nm decreased upon increasing concentration of added pyrophosphate which was accompanied by enhancement of a red-shifted broad emission band at 520 nm. The latter was due to the pyrophosphate-induced aggregation of conjugated polymer 19 and resulting intermolecular exciton coupling. On the other hand, phosphate anion chelated 19 showed no change in absorption and fluorescence spectra. The authors hypothesized that pyrophosphate bearing two anionic units could efficiently crosslink the polycationic chains of 19 thereby resulting in aggregation and aggregation-related observed spectroscopic changes.
Bai et al. prepared a 2,2'-bisimidazole incorporating conjugated polymer 20 whose fluorescence was effectively quenched upon formation of a complex with Cu$^{2+}$ through PET between the polymer backbone and Cu$^{2+}$ as the electron acceptor. The 20/Cu$^{2+}$ complex demonstrated good fluorescence enhancement upon titration with pyrophosphate anion due to the formation of a more stable complex of pyrophosphate/Cu$^{2+}$ thereby inhibiting the PET process. Of many common hard and soft anions, only pyrophosphate was found to trigger fluorescence enhancement of up to 9-fold within three minutes and the pyrophosphate detection limit was found to be 0.17 ppm.

The same authors also utilized poly[2,2'-bisimidazole-co-carbazole] conjugated polymer 21 as a platform for sensing cysteine. Upon addition of Ag$^+$ to the polymer, the complex 21/Ag$^+$ showed decreasing intensity of fluorescence emission at 416 nm and a simultaneous red shift of 40 nm. Two possible mechanisms were proposed to rationalize this observation: the first was PET from the polymer 21 to Ag$^+$ and the second was Ag$^+$-induced interchain aggregation resulting in an efficient FRET. The complex 21/Ag$^+$ was then used to detect cysteine since Ag$^+$ possesses strong association constant towards cysteine. The fluorescence ratio ($F_{416\text{nm}}/F_{456\text{nm}}$) increased 4 times upon addition of cysteine, and the detection limit was established at 90 nM. 21/Ag$^+$ complex showed good selectivity towards cysteine among 15 other amino acids, yet glutathione showed a similar fluorescence response.
Swager et al. demonstrated that a nonemissive thioether-containing poly(p-phenylene ethynylene) 22 displayed amplified turn-on fluorescence when exposed to hydrogen peroxide.\textsuperscript{63} Pristine 22 showed a low fluorescence quantum yield ($\lambda_{FL}$ 457 nm, $\Phi_{FL}$ 0.01), but it was increased by more than 49-fold upon the addition of an oxidizing agent H$_2$O$_2$. Low concentration of H$_2$O$_2$ (100 nM) was sufficient to trigger the fluorescence response from 22 in three hours, however excess amount of H$_2$O$_2$ lead to oxidizing the ethynyl linker thereby interrupting the $\pi$-electron conjugated system. Fluorescence lifetime studies of 22 showed that the increased $k_{FL}$ was due to the greater spatial overlap of the frontier molecular orbitals due to delocalization of the HOMO along the conjugated backbone and the increased $\tau_{FL}$ was due to the decrease in the rate if intersystem crossing.

Thomas and co-workers reported diaryltetracene-functionalized poly(fluorene-co-phenylene) 23 as a ratiometric fluorescent singlet oxygen ($^1$O$_2$) sensor.\textsuperscript{64} The pendant diaryltetracene can undergo [4+2] cycloaddition with $^1$O$_2$ to form endoperoxide species. Formation of these species can suppress excitation energy migration from the polymer backbone to diaryltetracene chromophore and therefore restore fluorescence of the polymer backbone. This resulted in drastic changes in the emission spectra, where a tetracene emission band at 512 nm steadily decreased whereas the polymer backbone emission band at 417 nm increased.
The authors also showcased an alternative route of the excitation energy migration interruption. In conjugated polymer 24, the \(^1\)O\(_2\) acceptor diethynyltetracene unit was electronically coupled to the excitation energy donor poly(p-phenylene ethynylene).\(^{65}\) Upon exposure to reactive singlet oxygen, fluorescence of the conjugated polymer backbone at 469 nm greatly increased. This was due to the oxidized tetracene chromophore no longer accepting excitation energy. Time-resolved fluorescence spectroscopy and excited-state lifetime studies found that the lifetime of 24 gradually increased with an increasing fraction of the oxidized tetracene.

![Diagram](image)

Rochat and Swager reported an n-type conjugated polymer poly(pyridinium p-phenylene) 25 that was designed as a sensor for electron rich bioanalytes.\(^{66}\) This sensor could detect concentration of caffeine as low as 25 ppm. Reduction of 48% in fluorescence intensity with electron rich indole units could be attributed to the quenching due to electron transfer from indole to the high ionization potential conjugated polymer. This was evaluated in Stern-Volmer
quenching experiments and fluorescence lifetime studies.\textsuperscript{67,68} Unexpectedly, other analytes resulted in an increase in emission intensity, thus suggesting a more complex fluorescence amplification mechanism. With its large hydrophobic pentiptycene unit, 25 could display some intermolecular hydrophobic interactions which could be interrupted by added aromatic analytes.

Swager and coworkers also demonstrated a pseudoratiometric polymer-on/dye-off mechanism for sensing cyclic ketone vapors.\textsuperscript{69} The loading of 0.5 wt. % of a squaraine dye in the thin film of poly(p-phenylene ethynylene) 26 facilitated excitation energy transfer from the polymer to the squaraine chromophore. The insignificant spectral overlap between emission of 26 and squaraine absorption indicated domination of the Dexter-type mechanism of energy migration and therefore the excitation energy transfer was extremely sensitive to the intermolecular distance and orientation. Introduction of cyclohexanone upon exposure of the thin film to the analyte vapor sterically disturbed and changed mutual positions of the conjugated polymer and squaraine thereby resulting in decreasing energy migration to the squarane chromophore, with overall decrease of squaraine fluorescence band and concomitant increase of the fluorescence emission from 26.

\begin{center}
\includegraphics[width=\textwidth]{chemical_structures.png}
\end{center}

A wide variety of analytes ranging from metal ions, small organic nucleophiles to large biomolecules can be categorized into four main fluorescence turn-on mechanisms, such as FRET, aggregation/deaggregation, suppression of ICT, and suppression of PET. FRET requires
sophisticated chromophore design to allow spectral overlap between energy donors and acceptors. Aggregation/deaggregation is highly dependent on the surrounding solvent molecules resulting in ratiometric fluorescence response. On the other hand, suppression of ICT/PET is usually operated through direct removal/neutralization of electron deficient quenchers from the polymer chains leading to fluorescent reminiscence of conjugated polymers without causing emission shift. While there is a continuous ongoing expansion of the research on chemosensor design based on the four conventional strategies, this dissertation will focus on a new concept towards the design of turn-on fluorescent sensors.

1.5 Research Focus

This dissertation focuses on the developing and studies of the higher energy gap paradigm as a general principle in designing turn-on amplifying fluorescent conjugated polymer sensors, as the previous developments of turn-on fluorescent conjugated polymer sensors were impeded due to the need for excessively sophisticated design in terms of energy level matching and the practical difficulty in materials synthesis. To demonstrate the practical utility of the higher energy gap paradigm, chapter 2 describes the development of an efficient turn-on amplifying fluorescent sensor for hydrogen sulfide — an important industrial and biomedical analytical target. From a fundamental standpoint, the higher energy gap paradigm demonstrated the importance of the through-bond (Dexter-type) mechanism for intramolecular energy transfer in conjugated polymers — still a widely debated issue.

In chapter 3, to further generalize and study in detail the higher energy gap paradigm, a conjugated polymer based amplifying turn-on fluorescence sensor for cysteine detection is described. In particular, it illustrated sensitivity of the higher energy gap mechanism even to very
small (about 0.1 eV) changes in HOMO-LUMO gap at the receptor side, which makes it a concept of choice for designing a broad variety of practically useful chemosensors.

Chapter 4 describes the development of a new concept towards preparation of precision conjugated polymer thin films via stepwise alternating polymerization. A series of immobilized alternating donor-acceptor poly(p-arylene ethynylene)s (PAEs) ultrathin films was prepared via grafted-from stepwise Sonogashira polymerization. This chapter exemplified stepwise metal-catalyzed polymerization as an efficient and economical approach to precision conjugated polymer films. The properties, structure and morphology of the thin films have been investigated using atomic force microscopy (AFM), grazing incidence X-ray scattering and neutron reflectometry, as well as optical spectroscopy and electrochemical methods.

1.6 References


CHAPTER 2. HIGHER ENERGY GAP CONTROL OF FLUORESCENCE IN CONJUGATED POLYMERS: \textit{TURN-ON} AMPLIFYING CHEMOSENSORS FOR HYDROGEN SULFIDE* 

2.1 Introduction

Signal amplification through photoexcitation energy (exciton) migration is a characteristic feature of conjugated polymer (CP) fluorescent sensory materials.\textsuperscript{1–3} Amplifying CP-based fluorescent chemo- and biosensors are attractive for sensing a broad variety of analytes including environmental hazards, explosives, and chemical warfare agents and biological species.\textsuperscript{4–9} Compared to small-molecule based sensors, they provide higher sensitivity, broader analyte detection range, and tenability of spectroscopic response characteristic. The large majority of the conventional amplifying CP-based fluorescent sensors utilize the effect of CP fluorescence quenching by electrodeficient anayltes via photoinduced electron transfer mechanism and therefore display a \textit{turn-off} response.\textsuperscript{10–15} On the other hand, sensors displaying \textit{turn-on} fluorescent response (i.e., increasing their fluorescent emission upon interacting with an analyte) are more desirable from a practical standpoint but are much more difficult to design. A typical scheme that allows achieving an amplified \textit{turn-on} response utilizes reversal of the CP fluorescence quenching phenomenon. In such a scheme, the CP fluorescence is initially diminished through the reversible coordination of a quenching moiety to the polymer conjugated backbone. A selective interaction of a target analyte with the quenching moiety shifts the equilibrium toward the anaylte-quencher complex, therefore restoring the CP fluorescence.\textsuperscript{16–18}

A related scheme utilizing fluorescent resonance energy transfer (FRET) effect for turn-on sensing of various non-quenching analytes (such as DNA and other biomolecules) has been also developed.\textsuperscript{19–21} Although useful and efficient, these approaches possess intrinsic limitation such as relative complexity of the sensing scheme, the need to employ chromophores with spectral characteristics matching the FRET requirements, and the necessity to functionalized one of the components of the sensor (or the CP itself) with a FRET acceptor fluorophore. An alternative (but less commonly used) approach to achieve amplified turn-on response is to incorporate an analyte-specific unit/chromophore as part of the polymer π-conjugated backbone.\textsuperscript{22} In dilute solution of CP sensors, the intramolecular excitation energy (exciton) transfer process occurs by the combination of through-space dipole-induced dipole (Förster-type) mechanism and through-bond electron exchange (Dexter-type) mechanism.\textsuperscript{23–25} Although the relative contribution of through-bond mechanism is often debated to be inessential compared to the contribution of the through-space mechanism,\textsuperscript{26,27} its major role has been demonstrated in some specific cases.\textsuperscript{28,29} Indeed, when the sensing unit is electronically coupled to the polymer π-electron system, the fluorescent behavior is controlled by the through-bond energy transfer (which does not depend on the spectral overlap which determines the efficiency of the FRET-based sensors) and therefore could provide a powerful general mechanism to achieve an amplified fluorescent response. On the basis of this consideration, we recently proposed a “higher energy gap” paradigm, where a turn-on amplification can be achieved via restricting exciton migration in the π-conjugated polymer backbone through generating a higher energy gap site upon reaction with an analyte.\textsuperscript{30} In this scheme, the receptor site is part of the CP π-conjugated system (Figure 2.1). Reaction with a target analyte results in a local increase in the energy (HOMO-LUMO) gap at that site, which creates a local “roadblock” that randomly migrating excitons in the polymer backbone cannot pass through.
via the through-bond mechanism. Although the intramolecularly migrating excitons can still pass through the “roadblock” sites via the through-space (Förster-type) mechanism, the major reduction in the efficiency of the through-bond contribution is expected to substantially shorten the exciton diffusion length. Such a restriction of the exciton diffusion length should reduce the probability of exciton nonradiative quenching due to decreasing its chance to encounter with intrinsically present quenching sites in the CP chain, such as conformational or structural defects, as well as transient defects such as triplet states, photogenerated free charge carriers, or charge-separated states, etc. Therefore, by the design, this is expected to result in an increasing intensity of the CP fluorescent emission, i.e., a pronounced turn-on effect. Importantly, the fluorescent intensity enhancement in this case originates in the CP π-electron system and therefore does not depend on whether the reaction with analyte produces a fluorescent or nonfluorescent chromophore—a major and universal advantage of the current approach over all the other previously developed schemes. Since the fluorescence enhancement via the “higher energy gap” mechanism directly related to the exciton migration in CP, it should produce a similar kind of signal amplification as with utilizing quenching-based turn-off fluorescent sensing schemes. In the present work, we demonstrate how the general “higher energy gap” principle can be employed to rationally design an efficient amplifying turn-on fluorescent sensor for H$_2$S detection.

Figure 2.1. Schematic diagram of the “higher energy gap” control of CP fluorescence: reaction with an analyte (red circle) creates a local higher energy gap site in the CP backbone which shortens the exciton diffusion length in the polymer, therefore causing an increase in the intensity of fluorescent emission.
Hydrogen sulfide (H\textsubscript{2}S) is an attractive target for the development of different types of sensors owing its significance in industrial toxicology, human physiology, pathology, and biomedicine.\textsuperscript{33–36} In particular, its presence at low (from nanomolar to micromolar) concentration in biological media has made it a popular target for the development of fluorescent chemosensors.\textsuperscript{37–43} However, none of the described H\textsubscript{2}S-responsive chemosensors were amplifying, whereas, due to the low concentration in physiological media, its detection would benefit from signal amplification provided by using the CP platform. Therefore, to demonstrate the power of the “higher energy gap” paradigm, we decided to apply it toward designing an amplifying fluorescent turn-on sensor for H\textsubscript{2}S. We also demonstrated the role of various structural factors in the design of the “higher energy gap” sensors and how the sensing performance of such systems can be rationally optimized.

2.2 Molecular Design and Synthesis Routes

In order to design an amplifying turn-on sensor based on the “higher energy gap” principle, one needs to choose a relatively low energy fluorescent polymer and functionalize it with an analyte-specific unit in such a way that the reaction with the analyte would generate a local increase in the CP backbone energy gap. Typically, for each analyte of interest there are a few reactions that satisfy this requirement. For H\textsubscript{2}S detection, we decided to use nucleophilic addition of hydrosulfide anion HS\textsuperscript{-} (form of H\textsubscript{2}S in the physiological conditions) to an electron-deficient double bond as such a reaction.\textsuperscript{44,45} Therefore, we proposed a CP sensor 2-P\textsubscript{1} which includes an H\textsubscript{2}S receptor unit that is a fusion between cyanine and naphthalene moieties (Scheme 1a). The naphthalene fragment of the receptor is part of the polymer’s poly(arylene vinlylene) \(\pi\)-conjugated backbone. The specific selection of poly(arylene vinlylene) conjugated polymer was dictated by the requirement to have a fluorescent CP with a relatively low energy gap as a main prerequisite.
for the “higher energy gap” design. Reaction of hydrosulfide anion with the cyanine moiety in 2-P1 results in an effective electronic “isolation” of this π-delocalized unit from the naphthalene moiety, therefore resulting in a substantial increase in the HOMO-LUMO gap at this local site, but without disrupting the overall π-conjugation in the polymer. Preliminary DFT computations (at the B3LYP/6-31G* level of theory) confirmed that the HOMO-LUMO gap of the receptor chromophore would increase from 2.84 to 4.12 eV after the reaction with HS⁻ due to the effective electronic isolation of the cyanine unit from the CP π-conjugated system (Figure 2.2). Therefore, this shall create a local higher energy gap site – an effective “roadblock” for excitons randomly migrating in the polymer π-conjugated backbone. Restricting the intramolecular exciton migration would result in an amplified turn-on fluorescent response.

Figure 2.2. DFT computational studies (B3LYP/6-31G*) of the HOMO-LUMO gap change in the cyanine receptor upon reaction with H₂S. The molecule was truncated for better computational efficiency. Computed HOMO and LUMO surfaces of the initial receptor indicate complete electronic delocalization over the entire molecule. Reaction with HS⁻ effectively electronically isolates the former cyanine unit resulting in the increasing energy gap of the π-conjugated moiety from 2.84 to 4.12 eV.
The polymer 2-P1 was functionalized with tri(ethylene glycol) (TEG) substituents in order to increase the polymer’s solubility in aqueous media (as H₂S detection is normally carried out in aqueous system). It was prepared using Suzuki coupling polymerization of the two properly functionalized monomers followed by postpolymerization installation of the cyanine moiety via Knoevenagel condensation and subsequent purification via reprecipitation and dialysis. Determination of the extent of fluorescent amplification in the polymer sensor 2-P1 required comparison with a corresponding small-molecules sensor analogue 2-M1 (Scheme 1b) which was also prepared using Suzuki coupling. Since the “higher energy gap” mechanism is based on blocking the intramolecular exciton migration in the CP π-conjugated backbone, it was expected to be significantly affect by the polymer conjugation length. In order to test this, through varying the polymerization conditions, we prepared two polymer 2-P1a and 2-P1b with different molecular weights—polymer 2-P1a with a number-average molecular weight $M_n$ 27.7 kDa and a shorter polymer 2-P1b with $M_n$ 15.5 kDa. We anticipated a much stronger response with the polymer sensor 2-P1a due to its higher conjugation length.

Scheme 2.1. (a) Mechanism of H₂S sensing through the formation of the higher energy gap site in the backbone of CP 2-P1; (b) structure of the small-molecule sensor 2-M1 and control CP 2-P2.
To further confirm the action of the “higher energy gap” mechanism in polymer 2-P1, we prepared a reference conjugated polymer 2-P2 (Mn 15.9 kDa) without the cyanine H₂S sensing moiety to test that the poly(arylene vinlyene) conjugated backbone itself would not produce spectroscopic response on H₂S addition (Scheme 2.1b).

2.3 Photophysical Properties and Fluorescent Amplification

The UV/vis absorption spectrum of the small-molecule sensor 2-M1 displayed an intense band at 324 nm and a smaller band at 447 nm, the latter possible due to the presence of the cyanine moiety (Figure 2.2, left). In contrast, the polymer 2-P1a displayed the main absorption band at 416 nm (bathochromic shift of 92 nm compared to the main band of 2-M1) which reflected substantial electronic delocalization in the polymer. No separate longer wavelength cyanine chromophore band was noticeable, although it could be overshadowed by the intense main CP band. Polymer 2-P1b (with a lower degree of polymerization) expectedly displayed a less bathochromically shifted absorption band, which reflected its apparently lower conjugation length (Figure 2.2, left). A noticeable shoulder at approximately 450 nm was indicative of the presence of the cyanine moiety chromophore. Similar to absorption spectra, fluorescence spectra also indicated a significant electronic delocalization in the cased of CP sensor 2-P1a and 2-P1b, which displayed broad band with a maximum at approximately 510 nm and a shoulder at 680 nm, possible due to the energy transfer to the lower energy cyanine chromophore (Figure 2.2, right). The overall fluorescent intensity of the polymers 2-P1a and 2-P1b was quite low, possible due to strong exciton quenching with the electron–deficient cyanine moieties.

We first tested the response of the small-molecule sensor 2-M1. Addition of 200 nM aqueous HS⁻ to a dilute 13.3 μM solution of 2-M1 in acetonitrile produced no detectable change in absorption spectrum and an approximately 2.5-fold enhancement in the integrated fluorescent
intensity $F/F_0$ (expressed as a ration of integrated intensities with and without added analyte).

Further increasing of the added HS$^-$ concentration up to 10 µM produced no change in the absorption spectra and resulted in the unexpected decrease of the fluorescent intensity (Figure 2.5). Overall, the small-molecule sensor 2-M1 demonstrated a rather insignificant turn-on fluorescent response and a narrow analyte detection range, therefore indicating its poor ability to act as an H$_2$S fluorescent sensor.

Figure 2.3. Normalized UV/vis absorption (left) and normalized fluorescence (right) spectra of CP sensor 2-P1a and 2-P1b and related compounds in acetonitrile solutions. Concentrations: 10.8 µM (2-P1a and 2-P1b), 13.3 µM (2-M1), and 10.0 µM (2-P2). Extinction coefficient (M$^{-1}$cm$^{-1}$): 32600 (2-P1a), 14800 (2-P1b), 46400 (2-M1), and 5500 (2-P2).

We then proceeded with studying the CP sensor 2-P1a designed using the “higher energy gap” principle. Adding increasing concentrations (from 200 nM to 0.1 mM) of aqueous HS$^-$ to a dilute 10.8 µM solution of 2-P1a in acetonitrile produced a substantial gradual increase of the fluorescent emission, up to 74-fold as an integrated intensity ration $F/F_0$ (Figure 2.4). The lack of a significant change in the absorption spectra as well as no substantial wavelength shift accompanying the fluorescence enhancement indicated only minor electronic perturbations in the extended π-electron conjugated system of 2-P1a upon reaction with HS$^-$, in agreement with our design based on the higher energy gap mechanism. Indeed, creating exciton “roadblocks” in the CP 2-P1a backbone via reaction of the cyanine receptor with HS$^-$ diminished exciton
intramolecular migration length and resulted in increasing fluorescent emission from the polymer. Overall, the polymer 2-P1a sensor demonstrated a significant turn-on response, with approximately 10 times higher detection sensitivity and a broad analyte detection range, all in a dramatic contrast with the poor sensing performance of the small-molecules analogue 2-M1 (Figure 2.5). Also in a good agreement with the “higher energy gap” mechanism, CP sensor 2-P1b, with a smaller degree of polymerization, displayed a much diminished turn-on sensing performance, with only a 30-fold maximal increase in $F/F_0$ and approximately 20 times lower sensitivity than the polymer 2-P1a (Figure 2.5). Indeed, the overall short conjugation length in 2-P1b was responsible for a more limited intramolecular exciton migration and thus a smaller fluorescent enhancement upon creating higher energy gap “roadblocks” in the CP backbone. That the “roadblocks” responsible for the turn-on fluorescence enhancement were created via the reaction of HS$^-$ with the cyanine moiety was clearly illustrated by the control polymer 2-P2 which showed absolutely no response on HS$^-$ addition (Figure 2.5).

Figure 2.4. Change in absorption (left) and fluorescence (right) spectra of a 10.8 μM solution of 2-P1a in acetonitrile upon addition of increasing concentration of H$_2$S (the spectra were acquired in 6 min after H$_2$S addition). The inset shows a photograph of the solution before and after addition of 100 μM HS$^-$ upon irradiation with a hand-held UV lamps.

When evaluating the role of the “higher energy gap” mechanism in controlling the turn-on fluorescent response in the present case, it is important also to discuss possible contribution of an
alternative mechanism based on fluorescence quenching by electron-deficient cyanine moiety in the CP sensors 2-P1a and 2-P1b. In principle, the cyanine units can potentially play the role of quencher of the polymer fluorescence, and the subsequent reaction with HS\(^-\) can essentially convert these electron-deficient quenchers to nonquenchers. A similar mechanism for the fluorescence control of a small-molecules organic fluorophores has been previously demonstrated.\(^{46-48}\) It is possible that such a quenching might be responsible for the initially low fluorescent intensity of the polymer 2-P1a,b, and reduction of this quenching via reaction with HS\(^-\) could be part of the mechanism for the observed fluorescence enhancement. However, the dominant role of this alternative mechanism in our case is hardly possible. Indeed, considering the high density of the “quenching” cyanine sites in the CPs (one cyanine group per repeating unit), and taking into account the “superquenching” mechanism based on intramolecular exciton migration to the quenching sites,\(^{1,4,16}\) reaction even of a large fraction of cyanine units with HS\(^-\) would still leave enough quenching sites on the CP chain to keep overall fluorescence low.

Figure 2.5. Change in integrated fluorescence intensity of acetonitrile solution of polymer 2-P1a, 2-P1b, and 2-P2, and monomer compound 2-M1 upon addition of increasing concentration H\(_2\)S. The intensity is expressed as a ration of integrated area of a fluorescence band at each H\(_2\)S concentration divided by the area of the fluorescent band in the absence of analyte \((F/F_0)\). The plot uses logarithmic scale for the H\(_2\)S concentration axis to illustrate the broad range (from nanomolar to millimolar) of detectable analyte concentrations in the case of polymer sensor 2-P1a.
Only when most cyanine sites have reacted with HS\(^{-}\) (i.e., at the very high H\(_2\)S concentrations) could one expect the fluorescence turn-on response, if this mechanism played any significant role. Thus, if the alternative mechanism dominated, the turn-on response at the very low H\(_2\)S concentration would not be observed. This directly contradicts our experimental observation of the broad detection range starting with nanomolar H2S concentrations and demonstrates the dominant role of the “higher energy gap” mechanism. Quantitative estimation of the relative contribution of the two mechanism is difficult and is beyond the scope of this work.

![Graph showing fluorescent response of various analytes](image)

**Figure 2.6.** Normalized integrated fluorescence intensity (as a ratio of integrated areas of fluorescent bands after and before addition of the analyte) of a 10.8 µM solution of polymer **2-P1a** in acetonitrile upon addition of various analytes.

For the practical applications of the sensor polymer **2-P1a**, it should display not only high sensitivity but also high selectivity toward the target analyte. Figure 2.6 shows fluorescent response of **2-P1a** to various potentially competing nucleophiles, including a model for reactive oxygen species (H\(_2\)O\(_2\)) and ubiquitous biological thiols (glutathione (GSH) and cysteine (Cys)). Although F\(^{-}\) and S\(_2\)O\(_3\)^{-} anions did cause a small increase of fluorescence, only H\(_2\)S stimulated a significant fluorescent response. Therefore, excellent selectivity of **2-P1a** coupled with its high
response sensitivity and broad analyte concentration detection range could make it an excellent \( \text{H}_2\text{S} \) detector.

**2.4 Conclusions**

We have developed a novel general principle for designing efficient amplifying turn-on fluorescent sensors based on the “higher energy gap” paradigm. The “higher energy gap” mechanism is based on the restricting intramolecular exciton migration in CPs by creating high-energy sites in the conjugated polymer backbone upon reaction with analyte. Fundamentally, the success of this paradigm illustrates the importance of the through-bond (Dexter-type) mechanism for intramolecular energy transfer in conjugated polymers—a point of controversial debates in the literature. From the broader materials design standpoint, this means that in order to achieve the best efficiency of the “higher energy gap” mechanism, one needs to use CPs with smaller energy gap, potentially even smaller than that provided by the poly(arylene vinylene) backbone used in the present work. From a general practical standpoint, the new concept described herein will enable simple design of a wide range of highly efficient amplifying turn-on fluorescent sensors and indicators, based on conventional analyte-selective reactions.

**2.5 References**


CHAPTER 3. FLUORESCENCE TURN-ON AMPLIFYING CONJUGATED POLYMER SENSORS FOR CYSTEINE DETECTION

3.1 Introduction

L-cysteine (Cys) is one of the most important and abundant sulfur-containing amino acids for building proteins in organisms as well as regulating antioxidant glutathione (GSH) levels inside cells.\(^1\),\(^2\) Deviated concentration of Cys in human body is connected to a variety of syndromes, e.g. hair depigmentation, edema, lethargy, liver damage, leucocyte loss, and Alzheimer’s disease.\(^3\)–\(^5\) Traditional methods for measuring Cys levels such as high performance liquid chromatography,\(^6\) mass spectrometry,\(^7\) and capillary electrophoresis\(^8\) require high instrumental expenses, long data collecting time, and destruction of samples. Importantly, these methods are not optimal for home testing or point-of-care emergency room. To understand the real role of Cys in physiological processes of eukaryotic cells, inorganic nanoparticles and organic fluorescent chemosensors have been developed for \textit{in vitro} and \textit{in vivo} examinations.\(^9\)–\(^12\) The investigation is significant yet difficult since other biothiols, e.g. GSH, H\(_2\)S, and homocysteine (Hcy), can sabotage the analysis. Specifically, concentration level of GSH in living cells is 1-10 mM whereas Cys level is orders of magnitude lower (at the \(\mu\)M level); furthermore, similarity between Hcy and Cys in terms of structure and reactivity renders them to react with the same receptor sites of the sensory molecules.\(^13\),\(^14\) Conventionally, development of the fluorescent chemosensors for assaying Cys is mainly based on its affinity toward heavy metal ions and strong nucleophilicity.\(^15\) Therefore, numerous organic reactions have been exploited, including Michael addition,\(^16\) cleavage of sulfonamide and sulfonate ester,\(^17\),\(^18\) cleavage of selenium-nitrogen bonds,\(^19\) cleavage of disulfide,\(^20\),\(^21\) and cyclization of aldehyde.\(^22\) It has been proven that N-terminal cysteine rapidly forms thiazolidine in the presence of aldehyde in biomimic solution.\(^23\) Strongin \textit{et al.} reported that xanthene derivative \(\textbf{I}\) containing free aldehyde as a receptor can efficiently monitor Cys and Hcy.
concentration down to µM level.\textsuperscript{22} Upon the addition of Cys, UV-vis spectrum of the xanthene derivative showed ratiometric behavior and the fluorescence of it was quenched. Barbas III and coworkers demonstrated that a small molecule sensor \textsuperscript{2} increased its fluorescence intensity after reacting with Cys, showing the moderate detection range from 100 ppm to 5000 ppm.\textsuperscript{24} Lin \textit{et al.} also published a ratiometric fluorescent sensor \textsuperscript{3} for Cys and Hcy detection.\textsuperscript{25} Composed of an electron rich phenanthroimidazole and electron deficient aldehyde moiety, the pristine small molecule sensor exhibited a fluorescence band at 519 nm, which was assigned as originating from intramolecular charge transfer. Upon cyclization with Cys or Hcy, the intensity of the intramolecular charger transfer band diminished which was accompanied by an intensity increase of a new emission band at 394 nm, originating from the phenanthroimidazole moiety. Hong \textit{et al.} prepared an ortho-hydroxy aldehyde-functionalized coumarin derivative \textsuperscript{4}, which acted as a fluorescence turn-on sensor for the detection of Cys and Hcy.\textsuperscript{26}

![Chemical structures of sensors](image)

In the aqueous solution (HEPES buffer, pH 7.4), the coumarin derivative showed very low fluorescence due to the photoinduced electron transfer from the coumarin to the aldehyde.
Formation of thiazolidine upon reaction of aldehyde with Cys greatly increased the fluorescence up to 100 times in intensity. The author proposed that the enhancement was resulted from the hydrogen bonding between the electron lone pair at the nitrogen of thiazolidine and the hydroxyl group on the coumarin. Generally, design of the biological Cys sensor molecules requires rather complex synthesis and precise adjustment of the electronic characteristics of the sensor (e.g. sensor design based on FRET energy migration mechanism requires achieving spectral overlap between emission of donor and absorbance of acceptor). In a previous study in our group, an anthraldehyde end-capped oligo(p-phenylene ethynylene) (OPE) structure was used to modulate excitation energy transfer through the formation of a thiazolidine moiety at the terminus of the π-conjugated oligomer (Figure 3.1).

Figure 3.1. (A) General structure of end-functionalized OPE and its immobilization on glass surface; (B) reaction of cysteine and a schematic diagram to show origin of the ratiometric fluorescent response.

These OPEs were self-assembled on a glass surface to form a monolayer-thick film. The OPE monolayer film was exposed to an aqueous solution of Cys at 90 ºC for 20 minutes to allow the analytes react with the aldehyde end-caps through cyclization. Therefore, the reaction with Cys
resulted in a gradual increase in the ratio of fluorescence intensity $F_{450}/F_{520}$, where $F_{450}$ refers to the anthracenyl end-group emission and $F_{520}$ is emission from the oligo($p$-phenylene ethynylene) core. The observed ratiometric fluorescence effect stemmed from raising the energy gap of the terminal acceptor fluorophore above the energy gap of the donor conjugated oligomer chromophore. This demonstrated the principle possibility to control excitation energy migration in conjugated oligomers (and polymers) by changing the HOMO-LUMO gap of one of the conjugated units. Recently, we proposed the novel concept of the “higher energy gap” controlled fluorescence amplification and demonstrated how it could be used in design of turn-on fluorescent sensors for the detection of organophosphorous warfare agent mimic diethyl chlorophosphate, and biochemical messenger hydrogen sulfide, respectively. The “higher energy gap” mechanism can be triggered when a target analyte interacts with a receptor along the conjugated polymer backbone and results in increasing the local HOMO-LUMO gap at that site. Unlike conventional fluorescence “turn-on” amplification approaches that often require elaborate synthesis and sophisticated specialized design, e.g. installation of a removable fluorescence quenching moiety or utilizing a scheme where a precursor strongly electronically coupled with polymer backbone would form a high emissive fluorophore upon an analytical reaction. In this part of the project, we decided to apply the “higher energy gap” concept towards the detection of Cys in aqueous environment, utilizing aldehyde reaction with cysteine. Although a number of fluorescent Cys sensors have been described in literature, none of them were amplifying, and therefore could not benefit from the ability to detect a broad range of Cys concentrations.

3.2 Molecular Design of the Cysteine Sensor

To design a “higher energy gap” controlled fluorescence turn-on amplifying conjugated polymer for achieving the Cys detection, poly(naphthylene vinylene) scaffold was employed as
the suitable low energy gap conjugated polymer platform. Similar scaffold has been previously successfully applied in design of amplifying sensors for organophosphate warfare agent mimics diethyl chlorophosphate and biomedical analyte hydrogen sulfide (described in the previous chapter). Among a variety of reported analytical reactions targeting Cys, we identified the reaction of an aldehyde functional group that can efficiently react with Cys to form a thiazolidine five-membered ring. In addition to the relatively simple and straightforward synthetic route, the cyclization to form thiazolidine can efficiently tune the local energy gap of the chromophore without affecting the overall π-electron conjugation in the polymer backbone. Preliminary DFT computational study (at the B3LYP/6-31G* level of theory) indicated that the formation of the thiazolidine unit would increase the HOMO-LUMO gap of the receptor chromophore from 4.01 to 4.15 eV (Figure 3.2). While such a small energy gap change (0.14 eV) was predicted, one can anticipate that, according to the “higher energy gap” mechanism, this could be sufficient to effectively restrict intramolecular exciton migration and result in the amplified turn-on fluorescent response.

3.3 Synthetic Routes for Cys Conjugated Polymer Sensor

The initial synthetic route developed to approach conjugated polymer sensors for Cys detection began from the alkylation of 2-S9 with 1,3-propansultone under basic condition in a solution of butanone-ethanol 1/1 mixture to generate water-soluble potassium sulfonate salt 3-1, which could be readily purified by precipitation from cold water to give the product in a moderate yield (56%). The water-soluble potassium sulfonate side chain was chosen since triethylene glycol monomethyl ether (TEG) group (used in the H2S sensor design) provided only limited solubility not sufficient to perform analytical measurements in aqueous media such as in phosphate-buffered saline (PBS) solution. The subsequent Suzuki polymerization of 3-1 and 2-S6 utilizing Pd(OAc)2
and XPhos and K$_3$PO$_4$ as base was carried out in aqueous phase (H$_2$O-THF 1/1) to give Cys polymer sensors **3-P1**, **3-P2**, **3-P3**, and **3-P4**, which were prepared by varying reaction time to achieve different degree of polymerization on the assumption of step-growth mechanism (8 hours for **3-P1**, 24 hours for **3-P2**, 48 hours for **3-P3**, and 72 hours for **3-P4**).

![Figure 3.2. DFT studies (at B3LYP/6-31G* level) of the HOMO-LUMO gap change in the aldehyde-functionalized receptor upon reaction with Cys. The molecule was truncated for better simulation efficiency. The computed HOMO and LUMO surfaces of the initial receptor indicate complete electronic delocalization over the entire molecule. Reaction with Cys effectively converted initial aldehyde into thiazoldine leading to the increasing energy gap of the $\pi$-conjugated system from 4.01 to 4.15 eV.](image)

All four crude polymers were purified via dialysis in deionized water for 2 days (using a membrane with 3.5 kDa cutoff for **3-P1**, and 8 kDa for other three polymers). We assume that using this approach we obtained polymer sensors with different conjugated backbone lengths. The accurate values of molecular weights of these polymers are yet to be determined as the polymers appear to show heavy intermolecular aggregation during GPC experiments (DMF as eluent). These experiments are currently in progress. Such aggregation indeed could stem from the insufficient solubilizing ability of TEG side chains. To compare the effect of fluorescent amplification in the longer $\pi$-conjugated systems with a much shorter system, small molecule reference **3-M1** was
prepared by Suzuki coupling of 3-1 and 2-S12, and the crude product was then purified by vigorously rinsing with THF and CH₂Cl₂ following by recrystallization from H₂O-EtOH 1/9 to give the product as a light greenish solid in 83% yield. The chemical structure was then elucidated by NMR and further supported by HR-ESI-MS (m/z calc.: 497.1423; found: 497.1427).

Scheme 3.1. Synthetic route to conjugated polymers for Cys detection (3-P1, 3-P2, 3-P3, and 3-P4) and the small molecule analog 3-M1.

3.4 Photophysical Characteristics and Fluorescent Amplification

We characterized all fluorescent Cys sensor polymers using UV-vis and fluorescence spectroscopies. All the studies were performed using solutions in 1X PBS in ultrapure water, and the analytical reaction with Cys was allowed to run at 50 ºC for 1 hour to reach the equilibrium. The UV-vis absorption spectrum of the small molecule 3-M1 showed a major absorption band at
316 nm with a second, weaker band centered at 400 nm. The latter can be assigned as appearing due to weak intramolecular charge transfer from naphthalene to aldehyde moiety (Figure 3.3). For pristine solutions of polymer sensor 3-P1, an absorbance band with two maxima at 337 and 394 nm with an onset at 521 nm was observed. For polymer sensor 3-P2, 3-P3, and 3-P4, with presumably higher degree of polymerization, all the UV-vis absorption spectra showed featureless intense broad bands. The significantly higher extinction coefficients indicated a possibility of the heavy aggregation occurring in solutions in the case of polymers with higher molecular weights. The fluorescence spectrum of 3-M1 showed a single band with maximum at 495 nm, whereas the conjugated polymer sensors 3-P1, 3-P2, 3-P3, and 3-P4 were almost non-emissive.

Figure 3.3. UV-vis absorption (normalized, left) and fluorescence (right, inset: normalized FL) spectra of polymer sensors 3-P1, 3-P2, 3-P3, and 3-P4 and small molecule reference 3-M1 in 1X PBS. Concentration: 6.25 µM (3-P1, 3-P2, 3-P3, and 3-P4), and 37.5 µM (3-M1). (Extinction Coefficients (M⁻¹ cm⁻¹): 3136 (3-P1), 67696 (3-P2), 128971 (3-P3), 136229 (3-P4), and 15750 (3-M1).

Based on general considerations of fluorescent properties of conjugated polymers, we expected that the extended π-conjugation along the polymer backbone should result in bathochromic shifts relative to the model sensor 3-M1 in both UV-vis and fluorescence spectra. However, the normalized fluorescent spectra of the polymer sensors, regardless of their molecular weights, exhibited the same position of the emission maxima as in the small molecule sensor 3-
M1. Upon addition of Cys, the small molecule reference 3-M1 showed a moderate gradual decrease in fluorescence intensity, and insignificant changes in UV-vis absorption spectra, even upon addition of large excess of Cys. The fluorescence intensity decrease (turn-off response) could probably be explained by the heavy atom effect from sulfur upon formation of thiazolidine moiety. Additionally, this could be attributed to the lower solubility of the thiazolidine derivative in the aqueous conditions, causing some material precipitating from the solution. Indeed, we observed accumulation of an insoluble solid from the measured samples after some time.

![Figure 3.4](image)

Figure 3.4. Changes in absorption (left) and fluorescence (right) spectra of a 37.5 µM solution of 3-M1 in 1XPBS upon addition of increasing concentrations of Cys.

On the other hand, copolymer 3-P1, 3-P2, 3-P3, and 3-P4 showed rather insignificant changes in UV-vis and fluorescence spectra upon addition of micromolar concentrations of Cys, yet the significant enhancement in fluorescence was observed upon addition of millimolar levels of Cys (Figure 3.5). The narrow Cys detection range could be attributed to the aggregation of the polymer chains in solution. Regarding the amplification ratio, as presented in Figure 3.6, 3-P1 showed an up to 30-fold amplification, and 3-P2, 3-P3, and 3-P4 showed smaller, 10 to 15 times, enhancements. The unexpected observation that the polymers with longer chains showed lower fluorescence amplification was contrary to the higher energy gap mechanism, as polymer sensors
with longer conjugated chain length were expected to show higher fluorescent amplification (see Chapter 2). This unexpected behavior could likely result from the poor solubility of the polymer in aqueous media, which induced intermolecular aggregation. Such aggregation would naturally be more prevalent in the case of longer macromolecular chains.

Figure 3.5. Changes in absorption (left) and fluorescence (right) spectra of a 6.25 µM solution of 3-P1, 3-P2, 3-P3, and 3-P4 in 1XPBS upon addition of increasing concentrations of Cys.
The intermolecular aggregation would create numerous close contacts between individual polymer chains which facilitate intermolecular energy transfer via the through-space (Förster-type) mechanism. The possibility of intermolecular energy migration would allow exciton “detour” of the local higher energy site in the conjugated backbone, thereby deactivating the higher energy gap mechanism. Indeed, intermolecular excitation energy transfer is considered to occur more efficiently as compared to the intramolecular pathway.

![Figure 3.6](image)

Figure 3.6. Change in integrated fluorescence intensity of polymers 3-P1, 3-P2, 3-P3, and 3-P4 solution in 1XPBS (6.25 µM), and small molecule 3-M1 (37.5 µM) upon addition of increasing concentrations of Cys. The intensity is expressed as a ratio of integrated area of a fluorescent band at each Cys concentration to the fluorescent band in the absence of analyte ($F/F_0$).

### 3.5 Conclusions

A series of conjugated polymers functionalized with electronically coupled aldehyde receptor for the detection of cysteine has been prepared. The featureless UV-vis spectra and high extinction coefficients indicated the polymers were highly aggregated in aqueous media. Upon addition of cysteine, the conjugated polymers exhibited substantial amplified fluorescent turn-on
response: sensor 3-P1 showed a 30-times enhancement in fluorescence intensity, and 3-P2, 3-P3, and 3-P4 showed 10 to 15-folds amplification, in the minimolar concentration range. This study demonstrated that the “higher energy gap” control mechanism for fluorescence turn-on amplification can be inhibited by intermolecular aggregation and, more importantly, is highly sensitive even to the small increase in local HOMO-LUMO gap at the reactive site. Further continuation of this work requires design and preparation of more water-soluble analogs of the conjugated polymer sensors, in order to minimize the effect of intermolecular aggregation. This work is ongoing.

3.6 References


CHAPTER 4. CONJUGATED POLYMER THIN FILMS WITH ORDERED MOLECULAR ORGANIZATION AND MORPHOLOGY BY STEPWISE SURFACE-INITIATED POLYMERIZATION

4.1 Introduction

Performance of conjugated polymers based electronic and optoelectronic devices greatly depends on intrinsic electronic properties of polymers and their organizations as bulk materials. While uniform alignments of conjugated polymer chains provide potential benefits for charge injection and charge transportation process, fine adjustment on it and further manipulation of mesoscale morphology in thin films remain a significant challenge. Currently, one most utilized solution-based approach is spin-casting that utilizes centrifugal force to randomly disperse polymer molecules onto substrates where the physical adsorbed thin films can be easily removed mechanically or chemically. Chemisorption of appropriate end groups, such as alkoxyisilane or phosphonate, of conjugated polymers (grafting-to) yields more robust thin films yet the polymer chains tend to form “mushroom” aggregates in solution prior to binding with surface. The “mushroom” architectures result in limited grafting density. On the other hand, surface-initiated polymerization (grafting-from) has been extensively harnessed in preparation of conjugated polymer thin films, such as polythiophene, polyfluorene, poly(p-phenylene), and poly(p-phenylene ethynylene). It is evident that this purification-free approach can produce very thick films with highly dense interchain packing. Furthermore, monomers used for surface-initiated polymerization do not require solubilizing side chains, which are major sites where oxidation, therefore, photo- and thermal degradation start. The solubilizing chains usually hinder the polymer packing resulting in effective “dilution” of the “semiconducting” fraction in bulk materials. However, this method is limited to chain-growth polymerization, and requires problematic immobilization of air-sensitive imitators, and sophisticated experimental settings. One
intermediary technique is to implement solution polymerization in the presence of a substrate decorated with functional groups which can take part in polymerization (grafting-through).\textsuperscript{16,17} Step-growth polymerization can occur spontaneously in the solution and on the surface thereby causing rough polymer thin films.

Alternating donor-acceptor conjugated copolymers allow one to chemically manage electronic properties of bulky materials since extent of orbital coherence between electron donating and accepting moieties can bring the band gap below 2.0 eV (a threshold for practical applications as semiconducting materials), as a result of lowering the required energy for the $\pi$-$\pi^*$ transition.\textsuperscript{18–21} Such low band gap conjugated polymers exhibit several promising redox and photoelectronic properties, e.g. broad and long wavelength absorption, solid-state charge transfer, and multiple charge states in a small potential window. Among various low band gap conjugated polymers, poly($p$-arylene ethynylene)s (PAEs) are especially attracting due to its unique linear rigid $\pi$-conjugated backbone and exceptional ability to transfer photoexcitation energy.\textsuperscript{22–27} Furthermore, relatively simple and straightforward synthesis of monomers, mild and highly reproducible polymerization conditions which can tolerate numerous functional groups, including sulfonate, amine, carboxylate, hydroxy, and biologically active ligands indeed facilitate the generalizing of PAEs.

In a previous study, our group demonstrated preparation of semiconducting thin films of poly(bithiophenetriazole fluorene) and poly(triazole fluorene) using surface-initiated stepwise click polymerization.\textsuperscript{28} The robustness, regioselectivity and chemoselectivity of Cu(I)-catalyzed acetylene-azide click reaction allowed to prepare highly organized anisotropic semiconducting polymer thin films with precise control of thickness and polymer molecular structure. However, the triazole units were not completely “conjugated” in a sense that they possess properties of
“cross-conjugated” units, and thus do not allow complete \( \pi \)-electron delocalization. In this chapter we describe our development, for the first time, of a simple and efficient procedure for the highly ordered truly conjugated immobilized poly(\( p \)-phenylene ethynylene) (PPE) thin films (Figure 4.1) consisting of precisely defined alternating donor-acceptor units using surface-initiated stepwise Sonogashira polymerization under mild reaction condition.

Figure 4.1. Chemical structures of surface-confined PPE polymer thin films prepared in this study.

**4.2 Synthetic Strategy towards Surface-Confinned PPE Thin Films**

The surface-confined initiator 4-1, triethoxy(4-iodophenyl)silane, was prepared from commercially available 1,4-diiodobenzene through halogen-lithium exchange reaction following by the quenching of organolithium compound with tetraethoxysilane, as shown in scheme 4-1. The initiator 4-1 was covalently immobilized onto the homogeneous inorganic oxide substrate to generate surface-immobilized self-assembled monolayer 4-SAM1. To prepare DMDM semiconducting polymer thin film, 4-SAM1 was immersed into a solution of 1,4-dimethoxy-2,5-diethynylbenzene (2), Pd(PPh\(_3\))\(_4\), and Cul in toluene/diisopropylamine at 40 °C for 30 minutes to obtain an ethynyl-terminated thin film (step A). After thoroughly rinsing with toluene under inert gas atmosphere to remove unbounded reactants, catalysts, and reaction byproducts, the ethynyl-terminated thin film was then immersed into a solution of 1,4-diiodo-2,5-dimethoxybenzene (5),
Pd(PPh$_3$)$_4$, and Cul in toluene/diisopropylamine at 40 °C for 30 minutes followed by vigorous washing with toluene and sonication for 3 minutes to obtain an iodo-terminated thin film (step B). The conjugated polymer thin film **DMDM** was prepared by alternation of steps A and B for 9 times, and exhibited robust chemical and mechanical tolerance. We further demonstrated the preparation of an n-type polymeric semiconductor **TFTF**, and low band gap conjugated polymer thin films **TFDM** and **DMBTD**, following the same approach and utilizing different diethynyl- and/or diiodo-monomers. While dimerization of diethynyl-functional monomers could occur in the presence of trace amount of Cu(I) and oxygen, neither self-coupling nor polymeric aggregates were observed during the entire reaction period.

Scheme 4.1. Preparation of semiconducting PPE thin films via stepwise surface-imitated Sonogashira polymerization.

Furthermore, for the best quality of the polymer films, the reaction solutions were prepared freshly at every single step, thereby maintaining the well-defined reactants concentrations, and minimizing formation of possible byproducts generated in reaction media over more extended reaction times.
4.3 Photophysical Characteristics of the Surface-Confined PPE Thin Films.

The evolution of the polymer thin films could be tracked by UV-vis absorption (Figure 4.2). The initiator surface-immobilized monolayer 4-SAM1 displayed a distinct UV-vis absorbance maximum at 239 nm corresponding to \( \pi-\pi^* \) transition of the phenyl group. We then measured the UV-vis progression of the thin films after each coupling cycle (one step A followed by one step B) at the iodo-termination step, in order to avoid potential oxidation of the ethynyl terminal group during the UV-vis measurements. In DMDM thin films, absorbance maximum (\( \lambda_{\text{abs}} \) 409 nm) showed a significant bathochromic shift (170 nm) accompanied by a linear increase in the absorption intensity: this appeared as a clear evidence for the extension of \( \pi \)-electron delocalization. Furthermore, the featureless long wavelength onset tailing beyond the detection range of the spectrometer indicated possible significant intermolecular electronic delocalization as a result of the close packed macromolecular chain arrangement on the surface. Likewise, TFTF thin film showed absorbance maximum at 351 nm with a red shift of 112 nm with respect to the initiator monolayer, and the linear increase of optical absorbance referred to the gradual growth of the polymer film. In the alternating donor-acceptor TFDM thin film, a broad absorption band featured a dual maximum at 330 and 405 nm, the latter can be assigned as a charge transfer band between the donor and acceptor moieties. Incorporating a stronger electron acceptor monomeric block 2,1,3-benzothiadiazole, semiconducting film DMBTD exhibited a broad absorbance with two maxima at 329 and 466 nm. It is worth noticing that the severe red-tailing of the long wavelength onset of the absorption band made an accurate determination of the energy band gap impossible. The energy band gaps of the four semiconducting polymer thin films determined using UV-vis spectroscopy and electrochemical studies are presented in Table 4.1.

The semiconducting polymers prepared by surface-initiated stepwise Sonogashira polymerization are expected to display uniform chain alignment in the direction perpendicular to the substrate surface and form a highly ordered densely packed bulk film. This is due to the highly rigid rod-like nature of the PPE macromolecules. To compare the polymer chain organization in surface-
confined thin films with the structure of thin films prepared by spin-casting method, the corresponding soluble analogs of TFDM and DMBTD, conjugated polymers 4-P1 (Mn 5.9 kDa; PDI 1.8) and 4-P2 (Mn 22.3 kDa; PDI 3.1) were synthesized by conventional Sonogashira polymerization. As the analog of TFDM, the solution of 4-P1 in CHCl3 showed a UV-vis absorbance maximum at 443 nm, which was assigned to charge transfer between electron donating and accepting units. The spin-casted 4-P1 thin film showed a similar absorbance signature with an additional shoulder at 500 nm and an onset-tail implying the increased intermolecular electronic delocalization. In sharp contrast, the UV-vis of TFDM showed a 40 nm hypsochromic shift relative to 4-P1 in CHCl3. In the case of DMBTD, a blue shift in absorption (40 nm) was observed compared to the soluble analog 4-P2 in toluene (λabs 524 nm), while its spin-casted thin film showed a 40 nm bathochromic shift.

![Figure 4.2](image_url)

Figure 4.2. UV-vis tracking of the growth of thin films of (a) DMDD and (c) TFTF; linear relationship of Eabs and cycle numbers of (b) DMDD and (d) TFTF.

These unusual results indicated that the alignment of polymer chains in the thin films prepared by surface-initiated polymerization was intrinsically different from the nanoscale organization of the thin films prepared by spin-casting of the soluble analogs. Bathochromic shifts
in absorption spectra are usually reported for spin-cast films because spin-casting procedure mechanically disperses isolated polymers on substrates leading to random molecular alignments. However, the substantial hypsochromic shifts in absorption spectra of TFDM and DMBTD thin films would be the consequence of a predominant normal to surface uniform alignment of the polymer backbones, in which the neighboring interacting \( \pi \)-conjugated units could form face-to-face stacked aggregates (similar to H-aggregates). Nevertheless, the broad bands with long wavelength tailing in absorption spectra of the thin films could stem from the mixture of face-to-face and head-to-tail aggregates (similar to J-aggregates).

Figure 4.3. UV-vis tracking of the growth of thin films of (a) TFDM and (c) DMBTD; linear relationship of \( E_{\text{abs}} \) and cycle numbers of (b) TFDM and (d) DMBTD.

4.4 Morphology and Uniformity of PPE Thin Films.

The surface morphology of TFDM and DMBTD thin films was analyzed by atomic force microscopy (AFM). Both thin films revealed a dense surface coverage with exceedingly smooth morphology. The thin film of DMBTD was composed of numerous uniform circular domains of average diameter 35 nm (Figure 4.6) with an approximate RMS roughness of 2.4 nm. The similarly shaped circular domains (60 nm in average diameter) were also observed in TFDM thin film
The formation of circular domains could stem from the polymer backbone structure and supramolecular alignment of the surface-confined polymer in the bulk thin films. The dense surface morphology with circular domains for the thin films was highly reproducible throughout this study.

Figure 4.4. (a) Superimposed UV-vis spectra of TFDM from solution (black), spin-casting (red) and grafting-from (blue); (b) Superimposed UV-vis spectra of DMBTD from solution (black), spin-casting (red) and grafting-from (blue).

Figure 4.5. Representative tapping mode AFM image of TFDM on quartz substrate. The topography (a) and phase (c) in 3 µm x 3 µm scan area; and the zoom-in topography (b) and phase (d) in 0.7 µm x 0.7 µm scan area.
To further evaluate nanoscale details and across-the-film structure of the semiconducting thin films, we prepared deuterated DMBTD (\textit{d-DMBTD}) thin films for neutron reflectometry studies. Neutron scattering is considered as a “soft” method, compared to X-ray scattering, and shows high sensitivity to deuterium element than hydrogen since they possess different scattering cross-section for neutrons. In the scattering length density (SLD) profile based on the best fitting model (Figure 4.X), we clearly observed the thickness of \textit{d-DMBTD} around 14.5 nm, which was in a good match with the theoretical value (13.2 nm) assuming the polymer chains were aligned normally to the substrate. The constant scattering length density (SLD) of approximately $2.8 \times 10^{-6}$ Å$^{-2}$ across 80% of the thin film thickness and the corresponding mass density of this high-density region calculated from this SLD value was 0.87 g cm$^{-3}$, indicating that the thin film consisted of densely packed \textit{d-DMBTD} polymer chains.

![Figure 4.6](image)

Figure 4.6. Representative tapping mode AFM image of DMBTD on quartz substrate. The topography (a) and phase (c) in 4 µm x 4 µm scan area; and the zoom-in topography (b) and phase (d) in 0.7 µm x 0.7 µm scan area.
4.5 Electrochemical Properties of PPE Thin Films.

All the PPE thin films were found remarkably stable and unaffected by prolonged sonication in organic solvents (CHCl₃, toluene, THF, acetone, MeOH, and EtOH) and water. Electrochemical studies of the thin films were conducted by cyclic voltammetry (CV) using thin films on ITO substrates as working electrodes and 0.1 M solution of n-Bu₄NPF₆ in acetonitrile as the supporting electrolyte. DMDM, TFTF, and TFDM showed irreversible oxidation or reduction features over the scans, but we were able to acquire information about their electronic energy levels before the degradation. As shown in table 4.1, the highest occupied molecular orbital energy level of DMDM (E_HOMO) was estimated at −5.99 eV against vacuum level. The lowest

Figure 4.7. Neutron reflectometry study of d-DMBTD thin film prepared by surface-initiated stepwise Sonogashira polymerization on a quartz substrate.

Table 4.1. Electrochemical Onset Potentials and Electronic Energy Levels of the Polymer Thin Films.

<table>
<thead>
<tr>
<th></th>
<th>ϕox(V vs Fc/Fc⁺)/ E_HOMO (eV)ᵃ</th>
<th>ϕred(V vs Fc/Fc⁺)/ E_LUMO (eV)ᵇ</th>
<th>E_g ec (eV)</th>
<th>E_g opt (eV)ᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>0.89/-5.99</td>
<td>n.a.ᵈ/−3.51ᶜ</td>
<td>n.a.ᵈ</td>
<td>2.48</td>
</tr>
<tr>
<td>TF</td>
<td>n.a.ᵈ/−6.52ᶜ</td>
<td>−1.17/−3.93</td>
<td>n.a.ᵈ</td>
<td>2.59</td>
</tr>
<tr>
<td>TFDM</td>
<td>1.05/−6.15</td>
<td>−1.37/−3.73</td>
<td>2.42</td>
<td>2.45</td>
</tr>
<tr>
<td>DMBTD</td>
<td>0.54/−5.64</td>
<td>−0.86/−4.24</td>
<td>1.40</td>
<td>1.87</td>
</tr>
<tr>
<td>4-P1</td>
<td>0.89/−5.99</td>
<td>−1.43/−3.67</td>
<td>2.32</td>
<td>2.38</td>
</tr>
<tr>
<td>4-P2</td>
<td>0.56/−5.66</td>
<td>−1.33/−3.77</td>
<td>1.89</td>
<td>2.04</td>
</tr>
</tbody>
</table>

ᵃCalculated according to the equation: E_HOMO = (ϕox + 5.1) (eV). ᵇCalculated according to the equation: E_LUMO = (ϕred + 5.1) (eV). ᶜEstimated from the onset wavelength of the absorption spectra: E_g opt = 1240/λonset. ᵈMeasurement on the cyclic voltammograms is unavailable.

²Calculated according to the equation: E_g opt = E_LUMO - E_HOMO.
unoccupied molecular orbital energy level of TFTF ($E_{\text{LUMO}}$) was determined at –3.93 eV. The $E_{\text{HOMO}}$ and $E_{\text{LUMO}}$ of TFDM were at –615 and –3.73 eV, respectively. These energy levels were in line with the values found from common semiconducting polymers. DMBTD gave $E_{\text{HOMO}}$ at –5.64 eV and $E_{\text{LUMO}}$ at –4.24 eV, and as expected, its energy band gap was slightly lower than 2.0 eV due to the strong donor-acceptor electronic coupling.

4.6 Conclusions

We have developed preparation of surface-confined semiconducting poly($p$-phenylene ethynylene) thin films via stepwise Sonogashira polymerization. This efficient and yet unprecedented approach allowed us to prepare PPE thin film with precise structure and controlled nanoscale morphology. All the thin films showed excellent chemical and mechanical tolerance. Both TFDM and DMBTD showed unusual hypsochromic shifts in $E_{\text{abs}}$ and long vibronic tails relative to their spin-casting counterparts, which was due to the highly dense uniform packing and face-to-face stacking of the $\pi$-conjugated units. AFM studies showed smooth morphology for DMBTF and good RMS roughness. Furthermore, the thickness of $d$-DMBTF thin film obtained using neutron reflectometry exhibited excellent match to the theoretical value assuming uniform normal-to-surface orientation of the conjugated macromolecules.

4.7 References


CHAPTER 5. EXPERIMENTAL SECTION

5.1 General Considerations

All reactions were performed under an atmosphere of dry nitrogen, except those that required Schlenk techniques, which were conducted under an atmosphere of ultrapure argon. Melting points were determined in open capillaries and were uncorrected. Chromatographic separations were carried out on silica gel (Sorbent Technologies, 60 Å, 40-63 µm, pH = 6.0-7.0) slurry packed into glass columns. Toluene, THF, DCM, ether, and hexane were dried by passing through columns of activated alumina and N,N-dimethylformamide (DMF) was dried through a column of molecular sieves both contained in a PS-400 Solvent Purification System from Innovative Technologies, Inc. The water content in the solvents was periodically controlled by coulometric titration on a Mettler Toledo DL 32 diaphragm-less coulometric titrator. Tetrabutylammonium hexafluorophosphate for electrochemical measurements was obtained from Aldrich and used after recrystallization from ethanol. Isopropylmagnesium chloride (2.0 M solution in THF) was purchased from Acros Organic, organometallic reagents were titrated with salicylaldehyde phenylhydrazone prior to use. High purity Pd(PPh₃)₄ was obtained from Stem, while all other reagents were obtained from Sigma-Aldrich and Alfa Aesar and used as received. Indium tin oxide (ITO) coated glass slides (25x75x1.1 mm³ polished float glass, 8-12 Ohm/sq. surface resistivity) were purchased from Delta Technologies, Ltd. 75x25 mm² sized polished rectangular quartz slides were purchased from Chemglass. ¹H NMR spectra were recorded at 400 MHz or 500 MHz unless otherwise indicated and were reported in parts per million downfield from tetramethylsilane. GPC analysis of polymers was performed with an Agilent 1100 chromatograph equipped with two PLgel 5 µm MIXED-C and one PLgel 5 µM 1000 Å columns connected in series, using THF as a mobile phase, and calibrated against polystyrene standard.
Dialysis purification was carried out using Spectrum Laboratories, Inc. Spectra/Por® dialysis tubing with MWCO 3.5kDa or 8kDa. DFT computations were performed under Windows version of Gaussian 09 computational package. UV-vis absorption spectra were recorded on an Agilent Cary 5000 UV-Vis-NIR spectrometer. Fluorescence studies were carried out using a PTI QuantaMaster4/2006SE spectrofluorimeter. Electrochemical measurements were carried out using a three-electrode system with Pt button working electrode (diameter 2mm), Ag/AgNO₃ non-aqueous reference electrode, and Pt wire counter electrode. Polymer thin film attached ITO substrate was used to replace Pt button working electrode when solid state measurement was necessary. The reference electrode was checked against ferrocene standard every time before and after the experiments were performed, and the measured potentials were corrected based on the Fc/Fc⁺ redox potential value. All experiments were carried out in 0.1 M Bu₄NPF₆ solution in THF or DCM as supporting electrolyte. High resolution mass spectra were obtained at the LSU Department of Chemistry Mass Spectrometry Facility.

5.2 Substrate Cleaning and Activation

Rectangular quartz slides (75 x 25mm) were cut using a ceramic cutting. The glass pieces were then washed with hexane, DMC, acetone, methanol, and then water for 30 minutes each under sonication. The slides were then thoroughly dried under a flow of nitrogen. The cleaned slides were placed in 20 mL scintillation vials and filled with freshly prepared piranha solution (7:3 H₂SO₄:30% H₂O₂) until the cover slip was submerged. (CAUTION: Piranha solutions are extremely corrosive and oxidizing. It must be prepared carefully due to exothermic mixing. Ensure that all contact surfaces are clean and free of any solvents from previous washings or other organic contaminants or else explosive conditions can be generated.) The vials were placed at rt for 1 h, then the slide removed and rinsed with copious amounts of Millipore-filtered water. The
rinsed slides were then dried under a flow of nitrogen and protected from dust and other particulates for 3 hours and were then ready for immobilization. ITO was activated using “basic piranha” (NH$_4$OH:H$_2$O$_2$:Water, 1:1:5) and prewashed using the same procedure as the glass slides. And placed at 75°C for 1, then the slide removed and rinsed with copious amounts of Millipore-filtered water. The rinsed slides were then dried under a flow of nitrogen and protected from dust and other particulates for 3 hours and were then ready for immobilization.

5.3 Atomic Force Microscopy

Samples were characterized with a model 5500 atomic force microscope (AFM) equipped with Picoscan v5.3.3 software (Agilent Technologies, Chandler, Az). Images were acquired using tapping mode in ambient condition. Nanoshaving was conducted using contact mode. Oxide-sharpened silicon nitride cantilevers with force constants ranging from 0.1 to 0.6 N/m were used for imaging (Veeco Probes, Santa Barbara, CA). Digital images were processed with Gwyddion open source software (version 2.9), which is supported by the Czech Metrology Institute.$^3$

5.4 Neutron Reflectometry

Neutron reflectivity measurements were performed at the Spallation Neutron Source Liquids Reflectometer (SNS-LR, Beamline 4B) at the ORNL. The reflectivity data were collected using a sequence of 3.25-Å-wide continuous wavelength bands (selected from 2.63 Å < $\lambda$ < 16.63 Å) and incident angles (ranging over 0.60° < $\theta$ < 2.71°), where $\lambda$ is the neutron wavelength and $\theta$ is the scattering angle. Using these settings, the momentum transfer, $q = (4\pi \sin \theta/\lambda)$ was varied over a range of 0.008 Å$^{-1}$ < $q$ < 0.22 Å$^{-1}$. Reflectivity curves were assembled by combining seven different wavelength and angle data sets together, maintaining a constant sample footprint and relative instrumental resolution of $\delta q/q = 0.023$ by varying the incident-beam apertures.
The reduced data consisted of absolute neutron reflectivity ($R$) vs. neutron momentum transfer $q$. Layers$^4$ and Motofit$^5$ software were used to fit the measured reflectivity curves, providing the reflectivity of a model scattering length density profile, which can be analyzed to determine the structure of the thin films. One or two layers were used to model the depth profiles of the films. The scattering length density, thickness, and roughness of each layer was freely varied in the fitting procedure. The quality of fit was gauged by minimizing $\chi^2$ between data and model reflectivity curves.

5.5 Supporting Information Associated to Chapter II

Figure 5.1. Change in absorption (left) and fluorescence (right) spectra of a 13.3 µM solution of the small-molecule sensor 2-M1 in acetonitrile upon addition of increasing concentrations of H$_2$S (the spectra were acquired in 6 min after H$_2$S addition).

Figure 5.2. Change in absorption (left) and fluorescence (right) spectra of a 10.8 µM solution of the lower molecular weight polymer sensor 2-P1b in acetonitrile upon addition of increasing concentrations of H$_2$S (the spectra were acquired in 6 min after H$_2$S addition).
Figure 5.3. Absorption (left) and fluorescence (right) spectra of a 10.0 µM solution of the control polymer 2-P2 in acetonitrile upon addition of increasing concentrations of H2S (the spectra were acquired in 6 min after H2S addition).

Figure 5.4. GPC elution traces for conjugated polymers 2-SP1a and 2-SP1b (precursors to sensor polymers 2-P1a and 2-P1b), and 2-P2. Experimental conditions: solvent THF, flow rate 0.7 mL min⁻¹, UV/vis absorbance detection at 450 nm.

5.6 Supporting Information Associated to Chapter IV

Figure 5.5. Photoluminescence spectra of TFDM, 4-P1 in DCM, and spin-casted 4-P1 (left), and DMBTD, 4-P2 in toluene, and spin-casted 4-P2.
Figure 5.6. Excitation spectra of TFDM, 4-P1 in DCM, and spin-casted 4-P1 (left), and DMBTD, 4-P2 in toluene, and spin-casted 4-P2.

Figure 5.7. Determination of TFDM film thickness by “nanoshaving” to reference the quartz substrate as a baseline in a scanning area of 3 µm x 3 µm; topography (left), phase (middle) and cursor profile (right).

Figure 5.8. Determination of DMBTD film thickness by “nanoshaving” to reference the quartz substrate as a baseline in a scanning area of 3 µm x 3 µm; topography (left), phase (middle) and cursor profile (right).
5.7 Synthetic Details

Scheme 5.1. Synthesis of polymer sensors 2-P1a-b, control polymer 2-P2, and small-molecule sensor 2-M1.
Scheme 5.2. Synthesis of small molecule building blocks, and soluble polymer 4-P1 and 4-P2.

Triethylene glycol monomethyl ether tosylate (2-S1)\(^6\) and 1,4-dibromo-2,5-bis(2-(2-(methoxyethoxy)ethoxy)ethoxy)benzene (2-S3)\(^7\) were prepared generally following established literature procedures.
1,4-bis(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)-2,5-((trimethylsilyl)ethynyl)benzene (2-S4). A solution of 1.75 g (17.8 mmol) of trimethylsilylacetylene, 2.53 g (4.46 mmol) of 2-S3, 103 mg (88.7 µmol) of Pd(PPh₃)₄, and 25.1 mg (0.131 mmol) of CuI in 150 mL of toluene/i-Pr₂NH (7:3) mixture was stirred in a sealed Airfree flask at 70 °C for 48 h. After allowing to cool down to room temperature, the reaction mixture was filtered through a glass filter, and the filtrate was concentrated in vacuo. The crude product was purified by column chromatography on silica gel (eluent ethyl acetate – CH₂Cl₂ (1:5)) to afford 2.15 g (78%) of 2-S4 as a colorless liquid that slowly solidified into a white waxy solid, Rᵣ 0.38. ¹H NMR (400 MHz, CDCl₃) δ 6.93 (s, 2H), 4.14 (t, J = 4.8 Hz, 4H), 3.89 (t, J = 4.8 Hz, 4H), 3.81 (t, J = 4.8 Hz, 4H), 3.70-3.66 (m, 8H), 3.57 (t, J = 4.8 Hz, 4H), 3.40 (s, 6H), 0.27 (s, 18H).

1,4-bis(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)-2,5-diethynylbenzene (2-S5). A solution of 317 mg (5.65 mmol) of KOH in 132 mL of MeOH/THF (1:3) was added into a solution of 1.41 g (2.35 mmol) of 2-S4 in 48 mL of THF, and the resulting solution was stirred at room temperature for 1 h. The reaction mixture was poured into water, extracted with dichloromethane (3 X 100 mL) and the organic fraction was washed with water (3 X 100 mL), dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (eluent ethyl acetate – CH₂Cl₂ (1:5)) to afford 990 mg (85%) of 2-S5 as a colorless liquid that slowly solidified into a white waxy solid at low temperature, Rᵣ 0.43. ¹H NMR (400 MHz, CDCl₃) δ 6.99 (s, 2H), 4.15 (t, J = 4.8 Hz, 4H), 3.86 (t, J = 4.8 Hz, 4H), 3.81 (t, J = 4.8 Hz, 4H), 3.70-3.66 (m, 8H), 3.57 (t, J = 4.8 Hz, 4H), 3.40 (s, 6H), 0.27 (s, 18H).

1,4-bis(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)-2,5-bis((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-1-yl)benzene (2-S6). A mixture of 800 mg (1.78 mmol) of 2-S5, 707 mg (4.09 mmol) of pinacolborane, and 46.0 mg (0.178 mmol) of ZrCp₂HCl in 20 mL of 1,2-dichloroethane
was stirred in a sealed Airfree flask at 65 °C for 72 h. After allowing to cool down to room temperature, the reaction mixture was poured into water, extracted with dichloromethane (3 X 100 mL), and the organic fraction was washed with water (3 X 100 mL), and dried over Na₂SO₄. Concentration in vacuo afforded crude product that was further purified by column chromatography on silica gel (eluent ethyl acetate – CH₂Cl₂ (1:1)) to afford 1.00 g (64%) of 2-S6 as a yellow sticky liquid that slowly solidified into a yellow solid at low temperature, Rf 0.30.¹ H NMR (400 MHz, CDCl₃) δ 7.70 (d, J = 18.6 Hz, 2H), 7.10 (s, 2H), 6.14 (d, J = 18.6 Hz, 2H), 4.15-4.09 (m, 4H), 3.90-3.84 (m, 4H), 3.77-3.74 (m, 4H), 3.71-3.66 (m, 12H), 3.56-3.54 (m, 4H), 3.37 (s, 6H), 1.30 (s, 24H).

3,6-Dibromo-2-hydroxy-1-naphthaldehyde (2-S9) was prepared starting from 6-bromo-2-naphthol following the previously described procedure.⁸

3,6-Dibromo-2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1-naphthaldehyde (2-S10). A mixture of 1.03 g (3.03 mmol) of 2-S9, 2.89 g (9.09 mmol) of 2-S1, and 1.26 g (9.09 mmol) of K₂CO₃ in 15 mL of DMF was stirred at 80 °C for 48 h. After allowing to cool to room temperature, the reaction mixture was poured into ethyl acetate, washed successively with H₂O (3 X 50 mL) and brine, extracted with ethyl acetate (3 X 50 mL), and dried over Na₂SO₄. After concentrating in vacuo, the crude product was purified by column chromatography (eluent ethyl acetate – hexane (1:5)) to afford 1.19 g (82%) of 2-S10 as a yellow liquid which was slowly solidified as a white waxy solid at low temperature, Rf 0.43.¹ H NMR (400 MHz, CDCl₃) δ 10.82 (s, 1H), 9.09 (d, J = 9.2 Hz, 1H), 8.22 (s, 1H), 7.90 (d, J = 2.1 Hz, 1H), 7.70 (dd, J₁ = 9.2 Hz, J₂ = 2.1 Hz, 1H), 4.36 (t, J = 4.8 Hz, 2H), 3.92 (t, J = 4.8 Hz, 2H), 3.73-3.71 (m, 2H), 3.68-3.64 (m, 4H), 3.54 (t, J = 4.8 Hz, 2H), 3.37 (s, 3H).
3,6-Dibromo-2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1-(2-methylprop-1-enyl)-naphthalene (2-S11). A solution of n-BuLi in hexanes (0.48 mL (0.768 mmol) of 1.6 M solution) was added dropwise into a solution of 327 mg (0.756 mmol) of isopropyltriphenylphosphonium iodide in 10 mL of THF at 0 °C. The resulting mixture was stirred at this temperature for 1 h, followed by addition of a solution of 200 mg (0.420 mmol) of 2-S10 in 10 mL of THF. The reaction mixture was stirred at room temperature for 16 h, then 10 mL of H₂O was added and the resulting mixture was stirred for 30 min. The solvents were removed under reduced pressure, and the residue was diluted with ethyl acetate and filtered through a pad of Celite. The filtrate was washed with water (3 X 100 mL), extracted with ethyl acetate (3 X 100 mL), dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (eluent ethyl acetate – hexane (1:5)) to yield 193 mg (92%) of 2-S11 as a clear liquid, Rf 0.52. ¹H NMR (400 MHz, CDCl₃) δ 7.91 (s, 1H), 7.85 (s, 1H), 7.69 (d, J = 9.0 Hz, 1H), 7.50 (d, J = 9.0 Hz, 1H), 6.35 (s, 1H), 4.05 (t, J = 5.1 Hz, 2H), 3.84 (t, J = 5.1 Hz, 2H), 3.75 (t, J = 5.1 Hz, 2H), 3.70-3.65 (m, 4H), 3.55 (t, J = 5.1 Hz, 2H), 3.38 (s, 3H), 2.03 (s, 3H), 1.49 (s, 3H).

(E)-4,4,5,5-tetramethyl-2-styryl-1,3,2-dioxaborolane (2-S12). A mixture of 500 mg (4.89 mmol) of phenylacetylene, 1.82 g (14.3 mmol) of pinacolborane, and 126 mg (0.489 mmol) of ZrCp₂HCl in 20 mL of 1,2-dichloroethane was stirred in a sealed Airfree flask at 65 °C for 72 h. After allowing to cool down to room temperature, the reaction mixture was poured into H₂O, extracted with dichloromethane (3 X 100 mL), washed with H₂O (3 X 100 mL), and dried over Na₂SO₄. Concentration in vacuo afforded crude product that was further purified by column chromatography on silica gel (eluent hexane – CH₂Cl₂ (3:1)) to afford 630 mg (59%) of 2-5 as a yellow sticky liquid, Rf 0.51 ¹H NMR (400 MHz, CDCl₃) δ 7.59 (d, J = 8.0 Hz, 2H), 7.42-7.29 (m, 4H), 6.19 (d, J = 18.4 Hz, 1H), 1.34 (s, 12H).
**Precursor polymer 2-SP1a.** A solution of 961 mg (4.53 mmol) of K$_3$PO$_4$ in 2 mL of H$_2$O was added into a solution of 297 mg (0.623 mmol) of 2-S10, 400 mg (0.566 mmol) of S6, 6.00 mg (26.7 µmol) of Pd(OAc)$_2$ and 27.1 mg (56.8 µmol) of XPhos in 10 mL of toluene, and the resulting solution was stirred in a sealed Airfree flask at 60 °C for 60 h. After allowing to cool down to room temperature, the reaction mixture was precipitated in MeOH resulting in 261 mg (60%) of 2-SP1a as a yellow sticky solid, $M_n$ 20.3 kDa, PDI 2.44 (GPC, vs. polystyrene). $^1$H NMR (400 MHz, CD$_2$Cl$_2$) δ 10.86 (s, 1H), 9.13 (s, 1H), 8.34 (s, 1H), 7.94 (s, 2H), 7.77-7.62 (m, 3H), 7.40-7.32 (m, 3H), 4.37-4.20 (m, 6H), 3.97-3.42 (m, 30H), 3.31-3.24 (m, 9H).

**Precursor polymer 2-SP1b.** A solution of 0.18 g (0.85 mmol) of K$_3$PO$_4$ in 1 mL of H$_2$O was added into a solution of 55 mg (0.12 mmol) of 2-S10, 74 mg (0.11 mmol) of 2-S6, 1.0 mg (5.3 µmol) of Pd(OAc)$_2$ and 42 mg (11 µmol) of XPhos in 5 mL of toluene, and the resulting solution was stirred in a sealed Airfree flask at 60 °C for 20 h. After allowing to cool down to room temperature, the reaction mixture was precipitated in MeOH resulting in 18 mg (20%) of 2-SP1b as a yellow sticky solid, $M_n$ 11.3 kDa, PDI 2.14 (GPC, vs. polystyrene). $^1$H NMR spectrum was similar to that of polymer 2-SP1a.

**Polymer 2-P1a.** A solution of 38 mg (49 µmol) of 2-SP1a, 0.45 g (1.5 mmol) of 1,2,3,3-tetramethyl-3H-indolium iodide, and 0.15 g (1.5 mmol) of KOAc in 2 mL of Ac$_2$O was stirred at 80 °C for 2 h. The reaction mixture was allowed to cool down to room temperature, and Ac$_2$O was removed in vacuo. The residue was dissolved in dichloromethane, and precipitated in hexane, and the resulting brown solid was washed successively with ethyl acetate and acetone. The crude product was further purified by dialysis from acetonitrile for 24 h to afford 38 mg (81%) of 2-P1a as a red sticky solid. The number average molecular weight $M_n$ 27.7 kDa was calculated based on $M_n$ of the precursor polymer 2-SP1a. $^1$H NMR (400 MHz, CD$_2$Cl$_2$) δ 8.95-8.91 (m, 1H), 8.40-8.33
(m, 2H), 8.12-8.02 (m, 3H), 7.81-7.58 (m, 7H), 7.48-7.27 (m, 3H), 4.33-4.28 (m, 9H), 3.99-3.43 (m, 30H), 3.31-3.24 (m, 9H), 2.03 (s, 6H).

**Polymer 2-P1b** was prepared following the procedure for polymer **2-P1a**, using 10 mg (13 µmol) of **2-SP1b**, 0.12 g (0.39 mmol) of 1,2,3,3-tetramethyl-3H-indolium iodide, and 38 mg (0.39 mmol) of KOAc in 1 mL of Ac₂O. This yielded 2 mg (17%) of **2-P1b** as a red sticky solid. The number average molecular weight $M_n$ 15.5 kDa was calculated based on $M_n$ of the precursor polymer **SP1b**. $^1$H NMR spectrum was similar to that of polymer **2-P1a**.

**Control polymer 2-P2.** A solution of 0.26 g (1.2 mmol) of K₃PO₄ in 1 mL of H₂O was added into a solution of 80 mg (0.16 mmol) of **2-S11**, 0.11 g (0.15 mmol) of **2-S6**, 1.7 mg (7.6 µmol) of Pd(OAc)₂ and 7.1 mg (15 µmol) of XPhos in 5 mL of toluene, and the resulting solution was stirred in a sealed Airfree flask at 60 °C for 72 h. After allowing to cool down to room temperature, the reaction mixture was precipitated in MeOH to afford 50 mg (42%) of **2-P3** as a yellow sticky solid, $M_n$ 15.9 kDa, PDI 2.24 (GPC, vs. polystyrene). $^1$H NMR (400 MHz, CD₂Cl₂) δ 8.09-6.95 (m, 10H), 6.45-6.34 (m, 1H), 4.30-3.28 (m, 45H), 2.06 (s, 3H), 1.58 (s, 3H).

**3,6-bis(2-Phenylethenyl)-2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1-naphthaldehyde (2-S13).** A solution of 401 mg (1.89 mmol) of K₃PO₄ in 2 mL of H₂O was added into a solution of 150 mg (0.315 mmol) of **2-S10**, 0.181 mg (0.79 mmol) of **2-S12**, 4.2 mg (16 µmol) of Pd(OAc)₂ and 17 mg (32 µmol) of BrettPhos in 5 mL of 1,4-dioxane, and the resulting solution was stirred in a sealed Airfree flask at 80 °C for 72 h. After allowing to cool down to room temperature, the reaction mixture was poured into H₂O, extracted with dichloromethane (3 X 50 mL), washed with H₂O (3 X 50 mL), and dried over Na₂SO₄. Concentration in vacuo afforded crude product that was further purified by column chromatography on silica gel (eluent ethyl acetate – CH₂Cl₂ (1:10)) to
afford 120 mg (73%) of 2-S13 as a yellow sticky solid, \( R_f 0.44 \). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 10.90 (s, 1H), 9.18 (d, \( J = 9.5 \) Hz, 1H), 8.30 (s, 1H), 7.86 (s, 1H), 7.85 (d, \( J = 8.0 \) Hz, 1H), 7.62 (d, \( J = 11.4 \) Hz, 2H), 7.59-7.53 (m, 3H), 7.44-7.38 (m, 4H), 7.34-7.28 (m, 3H), 7.26-7.25 (m, 2H), 4.24 (t, \( J = 4.3 \) Hz, 2H), 3.89 (t, \( J = 4.3 \) Hz, 2H), 3.75-3.73 (m, 2H), 3.71-3.69 (m, 2H), 3.65-3.63 (m, 2H), 3.54-3.52 (m, 2H), 3.36 (s, 3H).

**Small-molecule sensor 2-M1.** A solution of 60 mg (0.11 mmol) of 2-S13, 38 mg (0.13 mmol) of 1,2,3,3-tetramethyl-3H-indolium iodide, and 3 mg (32 \( \mu \)mol) of KOAc in 1 mL of Ac\(_2\)O was stirred at 80 °C for 2 h. The reaction mixture was allowed to cool down to room temperature, and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane, precipitated in hexane, and the resulting brown solid was washed thoroughly with ethyl acetate to afford 18 mg (24%) of 2-M1 as a red sticky solid. \(^1\)H NMR (400 MHz, CD\(_2\)Cl\(_2\)) \( \delta \) 8.89 (d, \( J = 16.4 \) Hz, 1H), 8.34 (s, 1H), 8.31(d, \( J = 8.7 \) Hz, 1H), 8.05 (d, \( J = 16.4 \) Hz, 1H), 8.01-7.99 (m, 2H), 7.78-7.76 (m, 1H), 7.70-7.61 (m, 7H), 7.56 (d, \( J = 16.4 \) Hz, 1H), 7.46-7.29 (m, 9H), 4.29 (s, 3H), 4.22-4.20 (m, 2H), 3.91-3.89 (m, 2H), 3.71-3.69 (m, 2H), 3.61-3.59 (m, 2H), 3.53-3.51 (m, 2H), 3.46-3.44 (m, 2H), 3.27 (s, 3H), 2.00 (s, 6H).

**Potassium 3-(3,6-dibromo-1-formynaphthalen-2-yloxy)propane-1-sulfonate (3-1).** A mixture of 26 mg (0.46 mmol) KOH in 1 mL of ethanol was added into a solution of 100 mg (0.3 mmol) of 9 in 3 mL of methyl ethyl ketone at 70 °C. A solution of 56 mg (0.46 mmol) 43 of 1,3-propanesultone in 1 mL of methyl ethyl ketone was added into the reaction mixture and stirred for overnight. After cooling down to rt, the reaction mixture was poured into acetone and white solid was precipitated. The white solid was collected after centrifuge and recrystallized from hot water to afford 77 mg (56%) of 10 as a white solid. \(^1\)H NMR (\( d_{6}\)-DMSO, 400 MHz): \( \delta \) 10.61 (s, 1H), 8.96 (d, 1H), 8.70 (s, 1H), 8.29 (s, 1H), 7.85 (d, 1H), 4.23 (t, 2H), 2.65 (t, 2H), 2.17 (m, 2H).

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Copolymer 3-P1, 3-P2, 3-P3, and 3-P4. Polymerization of copolymer 3-P1, 3-P2, 3-P3, and 3-P4 was performed using stock solutions of 120 mg (0.57 mmol) of K$_3$PO$_4$ in 12 mL of H$_2$O, 50 mg (0.07 mmol) of 2-S6 in 4 mL of THF, 32 mg (0.07 mmol) of 3-1 in 4 mL of THF, 0.3 mg (1.4 µmol) of Pd(OAc)$_2$ in 2 mL of THF and 1.7 mg (3.5 µmol) of XPhos in 2 mL of THF. Every stock solution was divided into four portions with equal volume, and resulting portions of reaction reagents and starting materials were then combined to give four reacting solutions with exactly the same concentrations. The reactions were conducted in 20 mL polycarbonate-capped glass vials at 55 °C in glove box. The reactions were then workup individually after 8 h for 3-P1, 24 h for 3-P2, 48 h for 3-P3, and 72 h for 3-P4. After allowing to cool down to room temperature, the reaction mixture was precipitated in acetone following by dialysis from deionized water for 2 days (3.5 kDa cutoff for 3-P1, and 8 kDa for others) to yield 10 mg of 3-P1 (72%), 5 mg of 3-P2 (36%), 6 mg of 3-P3 (44%), and 6 mg of 3-P4 (44%) as yellow sticky solid. $^1$H NMR of 3-P1 ($d_6$-DMSO, 400 MHz) δ 10.73 (s, 1H), 9.03 (s, 1H), 8.56 (s, 1H), 8.11-7.02 (br, 8H), 4.28-3.88 (br, 9H), 3.66-3.26 (br, 17H), 3.19-3.08 (m, 6H) 2.70 (s, 2H), 2.19 (s, 2H).

Small-molecule sensor 3-M1. A solution of 70 mg (0.33 mmol) of K$_3$PO$_4$ in 2 mL of H$_2$O was added into a solution of 25 mg (0.06 mmol) of 3-1, 38 mg (0.17 mmol) of 2-S12, 0.6 mg (2.8 µmol) of Pd(OAc)$_2$ and 3 mg (5.5 µmol) of XPhos in 2 mL of toluene, and the resulting solution was stirred in a sealed Airfree flask at 70 °C for 72 h. After allowing to cool down to room temperature, the reaction mixture was poured into acetone to form solid crude product. The solid was then vigorously washing with CH$_2$Cl$_2$ and THF to afford 22 mg (83%) of 2-S13 as a light greenish solid. $^1$H NMR ($d_6$-DMSO, 400 MHz) δ 10.72 (s, 1H), 9.04 (d, $J = 8.0$ Hz, 1H), 8.67 (s, 1H), 8.12 (s, 1H), 8.15 (d, $J = 12.0$ Hz, 1H), 7.73-7.67 (m, 4H), 7.65 (d, $J = 12.0$ Hz, 1H), 7.46-
7.40 (m, 6H), 7.36-7.31 (m, 3H), 4.18 (t, \( J = 8.0 \text{ Hz}, 2\text{H} \)), 2.68 (t, \( J = 8.0 \text{ Hz}, 2\text{H} \)), 2.17 (t, \( J = 8.0 \text{ Hz}, 2\text{H} \)). HR-ESI-MS (m/z calc.: 497.1423; found: 497.1427).

**Triethoxy(4-iodophenyl)silane (4-1)** was synthesized through a modified literature procedure.\(^1\) A solution of 0.7 mL (6.67 mmol of 1.85 M solution in THF) \( i-\text{PrMgCl} \) was added dropwise to a solution of 2.0 g (6.06 mmol) 1,4-diiodobenzene in 15 mL of anhydrous THF at \(-40 ^\circ\text{C} \) under \( \text{N}_2 \) atmosphere. The resulting mixture was stirred for 6 h to give a solution of \( (4\text{-iodophenyl})\text{magnesium chloride} \). The as prepared solution of \( (4\text{-iodophenyl})\text{magnesium chloride} \) was added dropwise via cannula to a solution of 3.12 g (30.3 mmol) Si(OEt)\(_4\) in 5 mL of anhydrous THF. The resulting solution was stirred at \(-40 ^\circ\text{C} \) for 1 h and then allowed to warm to rt overnight. The reaction mixture was diluted with 100 mL of \( \text{CH}_2\text{Cl}_2 \), washed with \( \text{H}_2\text{O} \), and extracted with \( \text{CH}_2\text{Cl}_2 \). The organic fraction was washed with brine, dried over \( \text{Na}_2\text{SO}_4 \), and concentrated in vacuo. The crude mixture was purified by Kugelrohr distillation (8 mm Hg, over temperature 130 \( ^\circ\text{C} \)) to give 0.67 g (32\%) of 4-1 as a clear liquid. \(^1\text{H NMR(\text{CDCl}_3, 400 MHz)} \delta 7.76 (d, \( J = 8.0 \text{ Hz}, 2\text{H} \)), 7.41 (d, \( J = 8.0 \text{ Hz}, 2\text{H} \)), 3.88 (q, \( J = 6.8 \text{ Hz}, 6\text{H} \)), 1.26 (t, \( J = 6.8 \text{ Hz}, 12\text{H} \)).

**1,4-Diiodo-2,5-dimethoxybenzene (4-5)** was prepared as described in the literature.\(^9\)

**1,4-Dimethoxy-2,5-bis(trimethylsilylethynyl)benzene (4-S2)**. 1.51 g (15.39 mmol) of (trimethylsilyl)acetylene was added to a mixture of 2 g (5.13 mmol) of 4-5, 119 mg (0.1 mmol) of \( \text{Pd(PPh}_3)_4 \) and 29 mg (0.15 mmol) of CuI in 150 mL of toluene - \( i-\text{Pr}_2\text{NH} \) (7:3). The resulting mixture was stirred at 70 \( ^\circ\text{C} \) in a sealed air-free flask for 48 h. After cooling down to rt, the mixture was concentrated in vacuo, and the crude product was purified by column chromatography on silica gel eluted with \( \text{CH}_2\text{Cl}_2 \) - hexane (1:3) to afford 1.36 g (90\%) of 4-S2 as a white solid. \(^1\text{H NMR(\text{CDCl}_3, 400 MHz)} \delta 6.93 (s, 2\text{H} \)), 3.85 (s, 6\text{H} \)), 0.28 (s, 18\text{H} \)).
1,4-Dimethoxy-2,5-diethynylbenzene (4-2). A solution of 1.3 g (3.93 mmol) of 4-S2 in 5 mL of CH₂Cl₂ was added into a mixture of 5.44 g (39.3 mmol) of K₂CO₃ in 80 mL of MeOH - CH₂Cl₂ (1:1). The resulting mixture was stirred at room temperature for 1 h. The mixture was filtered and the filtrate was collected and then diluted in 100 mL of CH₂Cl₂, washed with H₂O for three times, and then extracted with 100 mL of CH₂Cl₂ for three times. The resulting organic fraction was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel eluted with CH₂Cl₂ - hexane (1:3) to afford 695 mg (95%) of 4-2 as a white solid. ¹H NMR(CDCl₃, 400 MHz) δ 6.98 (s, 2H), 3.86 (s, 6H), 3.40 (s, 2H).

1,4-Diiodo-2,5-bis(trifluoromethyl)benzene (4-6) was synthesized through a modified literature procedure.¹⁰ 3 g (14.01 mmol) of 1,4-bis(trifluoromethyl)benzene was added into a mixture of 4.50 g (21.02 mmol) of NaIO₄, 6.98 g (42.03 mmole) of KI in 100 mL of H₂SO₄ at rt. The resulting mixture was stirred at 90 ºC for 16 h. After cooling to rt, the mixture was poured into ice, washed with H₂O, and filtered. The residual was collected, washed with Na₂S₂O₃ (aq), extracted with CH₂Cl₂. The resulting organic fraction was washed with brine, dried over Na₂SO₄, concentrated in vacuo, and recrystallized from hexane to afford 3.24 g (50%) of 4-6 as a white solid. ¹H NMR(CDCl₃, 400 MHz) δ 8.22 (s, 2H).

1,4-Bis(trifluoromethyl)-2,5-bis(trimethylsilyl)ethynylbenzene (4-S3) was prepared utilizing the same procedure as 4-S2. 1.7 g (97%) of 4-S3 as a white solid was acquired. ¹H NMR(CDCl₃, 400 MHz) δ 7.84 (s, 2H), 0.28 (s, 18H).

1,4-Bis(trifluoromethyl)-2,5-diethynylbenzene (4-3). A solution of 1.7 g (4.18 mmol) of 4-S3 in 5 mL of THF was added into a mixture of 5.78 g (41.82 mmol) of K₂CO₃ in 80 mL of MeOH - THF (1:1). The resulting mixture was stirred at room temperature for 1 h. The mixture
was filtered and the filtrate was collected and then diluted in 100 mL of CH₂Cl₂, washed with H₂O for three times, and then extracted with 100 mL of CH₂Cl₂ for three times. The resulting organic fraction was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel eluted with CH₂Cl₂ - hexane (1:3) to afford 987 mg (90%) of 4-3 as a white solid. ¹H NMR(CDCl₃, 400 MHz) δ 7.94 (s, 2H), 3.86 (s, 2H).

1,4-Dibromo-2,5-d₆-dimethoxybenzene (4-S4). A solution of 1 g (3.73 mmol) of 2,5-dibromohydroquinone, 1.35 g (9.33 mmol) of d₃-methyl iodide, and 2.06 g (14.92 mmol) of K₂CO₃ in 25 mL of DMF was stirred at 40 °C for 48 h. After cooling down to rt, the mixture was filtrated and the filtrate was purified by column chromatography on silica gel (DCM – hexane (3:1)) to yield 900 mg (80%) of 4-S4 as a white solid, Rf 0.34. ¹H NMR (400 MHz, CDCl₃) δ 7.10 (s, 2H).

1,4-d₆-Dimethoxy-2,5-bis(trimethylsilylethynyl)benzene (4-S5). 709 g (7.22 mmol) of (trimethylsilyl)acetylene was added to a mixture of 650 g (2.41 mmol) of 4-S4, 139 mg (0.12 mmol) of Pd(PPh₃)₄ and 46 mg (0.24 mmol) of CuI in 100 mL of toluene - i-Pr₂NH (7:3). The resulting mixture was stirred at 70 °C in a sealed air-free flask for 48 h. After cooling down to rt, the mixture was concentrated in vacuo, and the crude product was purified by column chromatography on silica gel eluted with CH₂Cl₂ - hexane (1:3) to afford 510 mg (63%) of 4-S5 as a white solid. ¹H NMR(CDCl₃, 400 MHz) δ 6.90 (s, 2H), 0.27 (s, 18H).

1,4-d₆-Dimethoxy-2,5-diethynylbenzene (4-4). A solution of 510 mg (1.52 mmol) of 4-S5 in 10 mL of THF was added into a mixture of 204 mg (3.64 mmol) of KOH in 40 mL of MeOH - THF (1:1). The resulting mixture was stirred at room temperature for 1 h. The mixture was filtered and the filtrate was collected and then diluted in 100 mL of CH₂Cl₂, washed with H₂O for three times, and then extracted with 100 mL of CH₂Cl₂ for three times. The resulting organic fraction was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was
purified by column chromatography on silica gel eluted with CH$_2$Cl$_2$ - hexane (1:3) to afford 276 mg (95%) of 4-4 as a white solid. $^1$H NMR(CDC$_3$, 400 MHz) δ 6.98 (s, 2H), 3.40 (s, 2H).

Alternating donor-acceptor type conjugated polymer 4-P1. 35 mg (0.064 mmol) of 1,4-Bis(tetradecyloxy-2,5-diethynyl)benzene, 30 mg (0.064 mmol) of 4-6, 1.4 mg (2 mmol%) of Pd(PPh$_3$)$_4$ and 0.24 mg (2 mmol%) of Cul in 10 mL of toluene - i-Pr$_2$NH (7:3). The resulting mixture was stirred at 70 °C in a sealed air-free flask for 72 h. After cooling down to rt, the mixture was poured into 50 mL of acetone to afford 30 mg (63% Mn 5.9 kDa PDI 1.8) of 4-P1 as a yellow solid. $^1$H NMR(CDC$_3$, 400 MHz) δ 7.98 (m, 2H), 7.02 (s, 2H), 4.05 (m, 4H), 1.87 (m, 4H), 1.43 (m, 44H), 0.89 (m, 6H).

Alternating donor-acceptor type conjugated polymer 4-7. 20 mg (0.036 mmol) of 1,4-Bis(tetradecyloxy-2,5-diethynylbenzene, 14 mg (0.036 mmol) of 4,7-diiodobenzo[c][1,2,5]-thiadiazole, 0.8 mg (2 mmol%) of Pd(PPh$_3$)$_4$ and 0.2 mg (2 mmol%) of Cul in 5 mL of toluene - i-Pr$_2$NH (7:3). The resulting mixture was stirred at 70 °C in a sealed air-free flask for 72 h. After cooling down to rt, the mixture was poured into 50 mL of acetone to afford 20 mg (80% Mn 22.3 kDa PDI 3.1) of 4-P2 as a red solid. $^1$H NMR(CD$_2$Cl$_2$, 400 MHz) δ 7.82 (s, 2H), 7.19 (s, 2H), 4.13 (t, 4H), 1.92 (t, 4H), 1.4 (m, 44H), 0.86 (m, 6H)

5.8 References


(3) *Gwyddion 2.9*; Metrology Institute, 2010.

(4) Layers Is an Excel Spreadsheet for Modeling Neutron Reflectivity Data Developed by John F. Ankner at the Oak Ridge National Laboratory.


CONCLUSIONS

A CP sensor **2-P1a** ($M_n \sim 27.7$ kDa) incorporates an H$_2$S naphthalene cyanine receptor which is part of the poly(arylene vinylene) $\pi$-conjugated backbone. Adding increasing concentrations (200 nM to 0.1 mM) of aqueous H$_2$S to a dilute solution of **2-P1a** in acetonitrile produced a substantial gradual increase of the fluorescent emission, up to 74-fold as an integrated intensity ratio $F/F_0$. The lack of a significant change in the absorption spectra as well as negligible wavelength shift accompanying the fluorescence enhancement indicated only the minor electronic perturbations in the extended $\pi$-electron conjugated system of **2-P1a** upon reaction with H$_2$S. The corresponding small-molecule sensor **2-M1** (prepared as a reference) produced no detectable change in the absorption spectrum and only an approximately 2.5-fold enhancement in $F/F_0$. Thus, the small-molecule sensor demonstrated a rather insignificant turn-on fluorescent response and a narrow analyte detection range. According to the “higher energy gap” mechanism, we expected that the sensing performance would be greatly affected by the polymer conjugation length, since this mechanism is based on restricting the intramolecular exciton migration in the CP $\pi$-conjugated backbone. Indeed, a CP sensor **2-P1b** ($M_n \sim 15.5$ kDa) with a lower degree of polymerization displayed a much-diminished turn-on sensing performance, with only a 30-fold maximum increase in $F/F_0$ and approximately 20 times lower H$_2$S sensitivity than the polymer **2-P1a**. This illustrated that the overall short conjugation length in **2-P1b** was responsible for a more limited intramolecular exciton migration and thus a smaller fluorescent enhancement upon creating higher energy gap exciton “roadblocks” in the CP backbone.

To prove that the “roadblocks” responsible for the turn-on amplified fluorescence enhancement were created via the reaction of H$_2$S with the cyanine moiety, we checked a control polymer **2-P2** which, as was expected, showed absolutely no response on H$_2$S addition. As an
excellent illustration of the power of the “higher energy gap” concept in fluorescent sensor design, the polymer sensor 2-P1a demonstrated a superior turn-on response, with approximately 10 times higher detection sensitivity and a broad analyte detection range, all in a dramatic contrast with the poor sensing performance of the small molecule analogue 2-M1.

To better understand the minimal requirements to the energy gap modulation for achieving an amplified fluorescence response in the “higher energy gap” mechanism, we turned to the cysteine sensor based on the reaction of an aromatic aldehyde with cysteine to form thiazolidine. Preliminary DFT (B3LYP/6-31G*) computations indicated that such a conversion would result in the energy gap change of approximately 0.1 eV. Such change normally would be too small to use in the design of conventional sensors, however it could be sufficient within the framework of the “higher energy gap” mechanism. Four CP sensors 3-P1, 3-P2, 3-P3, and 3-P4 were prepared by step-growth Suzuki polymerization using various reaction times to achieve different degree of polymerization. The goal was to obtain polymer sensors with different π-conjugation length. The accurate values of molecular weight of these polymers are yet to be determined as the polymers appear to show strong intermolecular aggregation during GPC experiments. Indeed, the featureless UV/vis spectra and unusually high extinction coefficients also indicated that the polymers were highly aggregated in aqueous media. Such aggregation could stem from the insufficient solubilizing ability of the side chains in aqueous environment.

Regarding the amplification ratio, CP sensor 3-P1 exhibited an up to 30-fold F/F₀ whereas 3-P2, 3-P3, and 3-P4 exhibited smaller, 10 to 15-fold enhancements, and all four polymers showed rather insignificant changes in absorption spectra after adding millimolar levels of cysteine. In contrast, small molecule sensor 3-M1 showed a moderate decrease in fluorescence intensity, due to the insufficient energy gap change.
Although, to our satisfaction, the “higher energy gap” based polymer sensors showed a reasonable turn-on response in the situation where a small-molecule sensor was not operational, we were not satisfied with a relatively small magnitude of the turn-on response. The less than desired sensing performance could be caused by poor solubility of the polymer in aqueous media, which induced intermolecular aggregation. Such aggregation could be responsible for numerous close contacts between individual polymer chains which would facilitate intermolecular exciton migration via the through-space mechanism. The possibility of intermolecular energy migration would allow an exciton “detour” of the local higher energy gap site in the CP backbone, thereby diminishing the effect of the higher energy gap mechanism.

Uniform alignment and improved molecular organization of CP chains in thin films could provide potential benefits for charge injection and charge transport processes in thin-film electronic and optoelectronic devices. However, fine adjustment and further fine-tuning of mesoscale morphology in films remain a significant and not yet solved challenge. In Chapter 4, we developed a precision stepwise Sonogashira polymerization to prepare a series of highly packed surface-immobilized PPE films with complete structural and morphological control. This method involves preparing thin films by adding one monomer repeating unit at a time using Sonogashira coupling protocol, and can be extended towards preparation of polymer chains of up to 20-30 repeating units.

In UV/vis spectra, all surface-confined PPE films showed significant bathochromic shift accompanied by a linear increase in the optical density: a clear evidence for the extension of the π-electron delocalization. It is worth noticing that the absorbance maxima in surface-confined precision polymers were hypsochromically shifted relative to the spectra of spin-cast reference films. This unusual (and previously unobserved) phenomenon could stem from the
interruption of continuous intramolecular $\pi$-conjugation caused by the alternating and uniform CP backbone twisting formed during the stepwise polymerization in the densely packed film. Indeed, atomic force microscopy studies on TFDM and DMBTD films exhibited highly dense packing and dramatic difference in morphology from the corresponding spin-cast films. To confirm the hypothesis of alternating backbone twisting, grazing-incidence wide-angle X-ray scattering studies showed that PPE films were amorphous and deficient of interchain $\pi-\pi$ stacking as a result of torsional twisting. From the neutron reflectometry (NR) studies, we obtained the thickness of a deuterated DMBTD film at 14.5 nm, which was in a good match with the theoretical value (13.2 nm) considering the polymer chains were aligned normally to the substrate. The mass density obtained the scattering length density in NR studies was 0.87 g cm$^{-3}$ indicating that the DMBTD film consisted of densely and uniformly packed polymer chains. In conclusion, the PPE films prepared by precision surface-confined stepwise Sonogashira polymerization showed unique and previously unobtainable thin-film morphology and polymer structure, which resulted in interesting electronic properties potentially well-suited for the applications in devices requiring enhanced charge transport across the film (e.g. organic light-emitting devices).
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APPENDIX B: $^1$H NMR SPECTRA OF KEY COMPOUNDS

Figure Appendix B.1. $^1$H NMR spectrum of compound 2-S5 (CDCl$_3$, 400 MHz).
Figure Appendix B.2. $^1$H NMR spectrum of compound 2-S6 (CDCl$_3$, 400 MHz).
Figure Appendix B.3. $^1$H NMR spectrum of compound 2-S9 (CDCl$_3$, 400 MHz).
Figure Appendix B.4. $^1$H NMR spectrum of compound 2-S10 (CDCl$_3$, 400 MHz).
Figure Appendix B.5. $^1$H NMR spectrum of compound 2-S11 (CDCl$_3$, 400 MHz).
Figure Appendix B.6. $^1$H NMR spectrum of compound 2-S12 (CDCl$_3$, 400 MHz).
Figure Appendix B.7. $^1$H NMR spectrum of compound 2-S13 (CDCl$_3$, 400 MHz).
Figure Appendix B.8. $^1$H NMR spectrum of compound 2-M1 (CD$_2$Cl$_2$, 400 MHz).
Figure Appendix B.9. $^1$H NMR spectrum of compound 2-SP1a (CD$_2$Cl$_2$, 400 MHz).
Figure Appendix B.10. $^1$H NMR spectrum of compound 2-SP1b (CD$_2$Cl$_2$, 400 MHz).
Figure Appendix B.11. $^1$H NMR spectrum of compound 2-P1a (CD$_2$Cl$_2$, 400 MHz).
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Figure Appendix B.1. $^1$H NMR spectrum of compound **2-P2** (CD$_2$Cl$_2$, 400 MHz).
Figure Appendix B.14. $^1$H NMR spectrum of compound 3-1 (d$_6$-DMSO, 400 MHz).
Figure Appendix B.1: H NMR spectrum of compound 3-P1 (d$_6$-DMSO, 400 MHz).
Figure Appendix B.1. $^1$H NMR spectrum of compound 3-M1 (d$_6$-DMSO, 400 MHz).
Figure Appendix B.1. $^1$H NMR spectrum of compound 4-1 (CDCl$_3$, 400 MHz).
Figure Appendix B.1.\(^1\)H NMR spectrum of compound 4-S1 (CDCl\(_3\), 400 MHz).
Figure Appendix B.19. $^1$H NMR spectrum of compound 4-2 (CDCl$_3$, 400 MHz).
Figure Appendix B.20. $^1$H NMR spectrum of compound 4-6 (CDCl$_3$, 400 MHz).
Appendix B.21. $^1$H NMR spectrum of compound 4-S3 (CDCl$_3$, 400 MHz).
Figure Appendix B.22. $^1H$ NMR spectrum of compound 4-3 (CDCl$_3$, 400 MHz).
Figure Appendix B.23. $^1$H NMR spectrum of compound 4-S4 (CDCl$_3$, 400 MHz).
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Figure Appendix B.27. $^1$H NMR spectrum of compound 4-P2 (CD$_2$Cl$_2$, 400 MHz).
VITA

Chien-Hung Chiang was born in 1984 in Taichung, Taiwan. He received his Bachelor of Science in Chemistry in June 2007 from Tamkang University in New Taipei, Taiwan. He began his research career in the lab of Professor Hsiu-Fu Hsu. In June 2009, he graduated with a Master of Science in Chemistry with a thesis titled, “Manipulation of Molecular Alignments of Liquid Crystalline Molecules by Self-Assembled Monolayers of Thiol-functionalized Discogens.” After serving for the R.O.C Air Force, he became a research assistant in the advanced organic materials laboratory in Tamkang University. His research interesting included supramolecular chemistry, organic photovoltaic, and amplification of chirality by sergeants and soldiers principle. In August 2012, he moved to Louisiana State University in Baton Rouge, Louisiana. He joined the laboratory of Professor Evgueni E. Nesterov and his major research contributed is the development of higher energy gap control of fluorescence in conjugated polymers and its application in sensing biomedical species, and surface-initiated stepwise polymerization. Chien-Hung is a candidate for the Doctor of Philosophy in Organic Chemistry which plan to be awarded in May 2018 with a dissertation entitled, “Higher Energy Gap Control of Fluorescence in Conjugated Polymers”.

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