Thermally Responsive Polymers and Their Applications

by

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Abstract

This thesis focuses on development of polymeric materials that can alter their functions according to temperature changes. We chose poly(N-isopropylacrylamide) (polyNIPA) as a platform, which phase-separates from water upon heating. The thermally responsive properties and applications of polyNIPA are introduced in Chapter One.

In Chapter Two, we described the synthesis of polyNIPA gels with an imidazole co-monomer and examined copper ion adsorption by the swollen (room temperature) and shrunken gels (60°C). The data analysis using a Langmuir adsorption isotherm indicates that the imidazole groups form 2:1 and 4:1 complexes with a copper ion in the swollen and shrunken gels, respectively, which suggests that thermal gel swelling and shrinking control the formation of multivalent Cu complexes by changing the distance among imidazole groups.

In Chapters Three to Six, the synthesis of polyNIPA-conjugated polymer block copolymers and their applications are described. Non-ionic water-soluble poly(phenylene-ethylenes) (PPEs) (Chapter Three) were used as conjugated polymer segments in the block copolymers. In a route to synthesis of the block copolymers, atom transfer radical polymerization (ATRP) and nitroxide-mediated radical polymerization (NMRP) of NIPA were developed. Incorporation of ATRP or NMRP initiators to the polymer ends of PPEs and the following polymerizations of NIPA were expected to provide tri-block copolymers with precise structures. The ATRP method produced pure polyNIPA with monodisperse and defined molecular weights (Chapter Four). However, endcapping of PPEs with an ATRP initiator (α-chloroamide) was not successful due to its instability to PPE polymerization conditions (Chapter Five). On the other hand, PPEs could be endcapped with a NMRP initiator (a tert-butyl nitroxide derivative), and the following NMRP of NIPA provided the tri-block copolymers (Chapter Six), phase-separate from aqueous solutions upon heating due to the polyNIPA aggregation.

In Chapter Six, we examined fluorescence resonance energy transfer (FRET) between a PPE-polyNIPA block copolymer and Rhodamine B (RhB) bound to polyNIPA. The RhB emission from the polymer precipitates produced by thermally induced phase-separation from the aqueous mixtures increased relative to that from the solutions, which indicates that thermal precipitation brought the PPE and RhB within the Förster radius of each other and induced FRET between the PPE and RhB.

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Table of contents

Abstract

Table of contents

Chapter 1: Introduction to thermally responsive polymers and their applications
  1.1 Thermally responsive poly(N-isopropylacrylamide) (polyNIPA)
  1.1.1 Applications
  1.1.2 PolyNIPA gels and their volume phase transitions
  1.2 Thesis overview
  1.3 References

Chapter 2: Cu ion adsorption and catalysis by polymer gels
  2.1 Introduction
  2.2 Experimental results and discussion
    2.2.1 Cu(II) ion adsorption
    2.2.2 Cross-linker concentration dependence
    2.2.3 Buffer effect
    2.2.4 Catalytic decomposition of H₂O₂
  2.3 Conclusion
  2.4 Experimental procedures
    2.4.1 Gel preparation
    2.4.2 Adsorption of Cu ions by the gels and determination of Cu concentrations
    2.4.3 Catalysis
  2.5 Appendix: Langmuir isotherm for gas-solid surface adsorption
  2.6 References

Chapter 3: Non-ionic water-soluble conjugated polymers
  3.1 Introduction
  3.2 Results and discussion
    3.2.1 Hydrophilic conjugated polymers
    3.2.2 Synthesis of hydrophilic monomers
    3.2.3 Polymerization
    3.2.4 Optical properties of Polymer A
    3.2.5 Nonionic hydrophilic thiophene-containing polymers
  3.3 Conclusion
  3.4 Acknowledgements
  3.5 Experimental procedures
    3.5.1 General
    3.5.2 Monomer synthesis
    3.5.3 Polymer synthesis
  3.6 References

Chapter 4: Atom transfer radical polymerization of N-isopropylacrylamide
  4.1 Introduction
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.1</td>
<td>Atom transfer radical polymerization (ATRP) and its mechanism</td>
<td>83</td>
</tr>
<tr>
<td>4.1.2</td>
<td>Previous studies on ATRP of acrylamide derivatives</td>
<td>85</td>
</tr>
<tr>
<td>4.1.6</td>
<td>ATRP design of NIPA</td>
<td>88</td>
</tr>
<tr>
<td>4.2</td>
<td>Results and discussion</td>
<td>89</td>
</tr>
<tr>
<td>4.2.1</td>
<td>ATRP of NIPA</td>
<td>89</td>
</tr>
<tr>
<td>4.2.2</td>
<td>Polymerization and purification</td>
<td>90</td>
</tr>
<tr>
<td>4.2.3</td>
<td>End-group analysis</td>
<td>91</td>
</tr>
<tr>
<td>4.2.4</td>
<td>Synthesis of PPE-graft-polyNIPA</td>
<td>94</td>
</tr>
<tr>
<td>4.3</td>
<td>Conclusion</td>
<td>96</td>
</tr>
<tr>
<td>4.4</td>
<td>Experimental procedures</td>
<td>97</td>
</tr>
<tr>
<td>4.4.1</td>
<td>General</td>
<td>97</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Initiators</td>
<td>97</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Polymerization</td>
<td>99</td>
</tr>
<tr>
<td>4.4.4</td>
<td>Synthesis of conjugated polymer-graft-polyNIPA (Polymer C)</td>
<td>100</td>
</tr>
<tr>
<td>4.5</td>
<td>References</td>
<td>104</td>
</tr>
</tbody>
</table>

Chapter 5: Synthesis of thermally responsive poly(phenylene-ethynylene) via atom transfer radical polymerization of N-isopropylacrylamide | 105 |

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Overview</td>
<td>106</td>
</tr>
<tr>
<td>5.1.1</td>
<td>Thermally responsive conjugated polymers</td>
<td>106</td>
</tr>
<tr>
<td>5.1.2</td>
<td>Block copolymerizations</td>
<td>108</td>
</tr>
<tr>
<td>5.2</td>
<td>Results and discussion</td>
<td>109</td>
</tr>
<tr>
<td>5.2.1</td>
<td>PPE macrorinitiator</td>
<td>109</td>
</tr>
<tr>
<td>5.2.2</td>
<td>PolyNIPA end-capped PPE</td>
<td>113</td>
</tr>
<tr>
<td>5.3</td>
<td>Conclusion</td>
<td>116</td>
</tr>
<tr>
<td>5.4</td>
<td>Experimental procedures</td>
<td>118</td>
</tr>
<tr>
<td>5.4.1</td>
<td>General</td>
<td>118</td>
</tr>
<tr>
<td>5.4.2</td>
<td>ATRP initiator/end-cap groups</td>
<td>118</td>
</tr>
<tr>
<td>5.4.3</td>
<td>Block copolymerization conditions</td>
<td>120</td>
</tr>
<tr>
<td>5.4.4</td>
<td>Fluorescence measurements</td>
<td>121</td>
</tr>
<tr>
<td>5.5</td>
<td>Reference</td>
<td>124</td>
</tr>
</tbody>
</table>

Chapter 6: Thermally responsive fluorescent conjugated polymers | 125 |

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Overview</td>
<td>126</td>
</tr>
<tr>
<td>6.1.1</td>
<td>Thermally responsive conjugated polymers</td>
<td>126</td>
</tr>
<tr>
<td>6.1.2</td>
<td>Nitroxide-mediated radical polymerization (NMRP)</td>
<td>127</td>
</tr>
<tr>
<td>6.2</td>
<td>Results and discussion</td>
<td>130</td>
</tr>
<tr>
<td>6.2.1</td>
<td>Synthesis of PPE-polyNIPA block copolymers</td>
<td>130</td>
</tr>
<tr>
<td>6.2.2</td>
<td>Poly(phenylene-bithiophene) (PPBT)</td>
<td>135</td>
</tr>
<tr>
<td>6.2.3</td>
<td>Thermally induced precipitation</td>
<td>137</td>
</tr>
<tr>
<td>6.3</td>
<td>Conclusions</td>
<td>156</td>
</tr>
<tr>
<td>6.4</td>
<td>Acknowledgements</td>
<td>158</td>
</tr>
<tr>
<td>6.5</td>
<td>Experimental procedures</td>
<td>159</td>
</tr>
<tr>
<td>6.5.1</td>
<td>General</td>
<td>159</td>
</tr>
<tr>
<td>6.5.2</td>
<td>NMRP initiator/end-cap, 3</td>
<td>159</td>
</tr>
<tr>
<td>6.5.3</td>
<td>Polymerization of PPE (macrorinitiator) 6</td>
<td>160</td>
</tr>
<tr>
<td>6.5.4</td>
<td>Polymerization of PPBT 8</td>
<td>161</td>
</tr>
<tr>
<td>6.5.5</td>
<td>General procedure of NMRP of NIPA with PPE or PPBT</td>
<td>161</td>
</tr>
</tbody>
</table>
6.5.6 Synthesis of RhB-polyNIPA IV 162
6.5.7 Synthesis of PPE-RhB-polyNIPA V 162
6.5.8 General protocol for thermal precipitation 163

6.6 References 167

Acknowledgements 168

Curriculum Vitae 169
Chapter 1: Introduction to thermally responsive polymers and their applications

Abstract: In this thesis, thermally responsive poly(N-isopropylacrylamide) has been used in the applications of molecular capture and controlled aggregation of fluorescent conjugated polymers in water.
1.1 Thermally responsive poly(N-isopropylacrylamide) (polyNIPA)

Poly(N-isopropylacrylamide) (polyNIPA), synthesized by free radical polymerization of NIPA, has been recognized as a thermally responsive material, and applications have been explored for chemical and biotechnological industries. PolyNIPA exhibits a unique thermally induced phase separation behavior in water.¹ An aqueous polyNIPA solution is homogeneous at room temperature with the polymer chains isolated and extended. However, above 31°C, the solution suddenly becomes cloudy due to the aggregation of the polymers, and precipitation is generally observed. This phase separation is reversible, and can be induced by slight changes of temperature around the transition temperature (31°C).

![NIPA](image1)

![PolyNIPA](image2)

The mechanism of the phase separation of polyNIPA is generally explained in terms of hydration/dehydration of the polymer chains. Water molecules are hydrogen-bonded to the amide groups of polyNIPA at room temperature, and this hydration keeps the polymer chain in solution. At elevated temperatures, increased random thermal motion of the molecules weakens the hydrogen-bonds, and the water tends to diffuse away from the polymers, thereby decreasing the hydration of the polymer chains. The hydrophobic character of polymer chains becomes dominant and causes polymer precipitation. This phenomenon resembles heat denaturation of proteins. At high temperatures, the protein structure fluctuates exposing the hydrophobic domains to the water interface, which induces aggregation of proteins. However, unlike polyNIPA, the
interaction between proteins is so strong that the aggregation is irreversible and the proteins are no longer active.

The phase separation temperature (lower critical separation temperature, LCST) of polyNIPA is determined by the balance between the hydrophilicity (ability to hydrogen bond with water) and the hydrophobicity (of the alkyl chains on the amide). Indeed, according to this principle, the LCST can be experimentally tuned by a random copolymerization of hydrophilic/hydrophobic monomers, and for example, random copolymers of acrylamide (AAm) (hydrophilic monomer) and NIPA exhibited higher LSCT than a homopolymer of NIPA.\textsuperscript{2,3,4} Additionally, the LCST of the copolymers increases with increasing molar fraction of the AAm comonomer.\textsuperscript{2} On the other hand, incorporation of hydrophobic monomers such as N-alkylacrylamide to the copolymers gives lower LCSTs.\textsuperscript{2} The presence of additives can also affect LCST, and for example, the addition of a salt lowers the LCST by increasing hydrophobic nature of the polymer due to a salting-out effect.\textsuperscript{5,6} In the presence of surfactants, LSCT increases because of solubilization of the hydrophobic moieties by surfactant micelles, leading to an increase of polymer hydrophilicity.\textsuperscript{7,8} These tunable nature of LCST allows us to design polyNIPA-based materials that are tailored to operate in specific conditions such as those encountered in physiological applications.\textsuperscript{9}

The LCST behavior of polyNIPA is characterized by means of a cloud point measurement.\textsuperscript{10} A cloud point of a polyNIPA aqueous solution is determined by monitoring a turbidity of a polyNIPA solution visually\textsuperscript{4} or an optical density\textsuperscript{6} at fixed wavelength using UV-vis spectrometer as a function of temperature. Other methods are
also used to characterize the phase separation: differential scanning calorimetry,\textsuperscript{5,11} light scattering,\textsuperscript{12} and fluorescence\textsuperscript{13,14}.

1.1.1 Applications

Thermally induced phase separation of polyNIPA has been utilized to (1) regulate chemical reactions,\textsuperscript{15} (2) capture molecules through bioconjugates,\textsuperscript{16} and (3) deliver polymeric carriers to targeted tumors\textsuperscript{9}.

(1) Bergbreiter \textit{et al.} reported that a polyNIPA supported catalyst could be recovered from aqueous reaction mixtures by heating, and that catalytic activities were turned on and off as the temperature was cycled.\textsuperscript{15} One such example is Rh (I) ionically bound to a polyNIPA derivative that catalyzes hydrogenation of allyl alcohol in water. Hydrogen uptake was observed at 22°C, where the polymer was in solution and thereby the reaction is rapid. At 55°C, the hydrogen uptake stops due to phase separation of the polymer catalyst. Not only could catalytic activity of the polymer-bound catalyst be turned on/off by changing temperature, but also the catalyst could be recovered by precipitation of the polymer from the reaction mixture.

(2) Hoffman and coworkers demonstrated how bioconjugates of polyNIPA could be used to regulate molecular binding to proteins\textsuperscript{17}, recover enzymes\textsuperscript{18}, and separate the target molecules\textsuperscript{16,19,20} with temperature changes. Bioconjugates of traditional polymers such as polyethylene glycol have been used to simply solubilize proteins in water.\textsuperscript{21} However, polyNIPA-based bioconjugates are more functional and have provided the new technological applications that utilize the thermally responsive properties. Monji and Hoffman proposed a new immunoassay technique using antibodies conjugated to
polyNIPA.\textsuperscript{16} In general, homogeneous immunoassays are easy to perform and have the advantages of producing efficient reactions with rapid kinetics. However, interferences are problematic, and the reagents and analytes are limited to low molecular weight species, which depresses the sensitivity of this type of assay. In fluorescence-based detection schemes, the signals from labeled reagents need to be distinguishable depending on whether they are bound and unbound. In the case of heterogeneous immunoassays using a solid interface such as enzyme-linked immunosorbant assay (ELISA), fewer interferences and higher sensitivity are obtained because of the efficient separation of other analytes and unbound reagents by washing. Similar separations are not possible in homogeneous assays. Since the unbound fluorescent labels can be removed, the fluorescent signals of bound labels need not be distinguishable from those of unbound labels, thereby providing another advantage over homogeneous assays. The disadvantages of heterogeneous assays are slow kinetics due to limits of the molecular diffusion on the solid surface that require long incubation times as well as extensive washing procedures that increase the time and cost. Non-specific adsorption of analytes also plagues heterogeneous assays. Antibodies conjugated to polyNIPA that precipitated out of water above 31°C and could be re-dissolved by cooling offer a chance to take advantages of both heterogeneous and homogeneous assays.\textsuperscript{16} This reversible solubility change allows easy separation of the bound immuno complex to the polymer from solution. This method provides incubation in homogeneous solution for optimal antigen-antibody interactions, facile isolation of analytes, and the concentration of labeled reagents to enhance the sensitivity. This assay principle was applied to sandwich immunoassays, wherein an antibody-conjugate of polyNIPA was incubated with an
antigen in a solution containing a second fluorescent dye-labeled antibody.\textsuperscript{16,19} These two antibodies bind different epitopes of the antigen and form an immuno sandwich complex. This immuno complex bound to polyNIPA could be precipitated out of the solution by heating and concentrated by centrifugation. After multiple washings to completely remove the unbound antibody, the polymer was re-dissolved in a small quantity of water by cooling, and the amount of the labeled antibody (immuno complex) was determined by measuring the fluorescence.

(3) Meyer et al. reported a new thermal targeting method using poly(NIPA-acrylamide) copolymers to deliver polymeric carriers to tumors.\textsuperscript{9} The LCST of the copolymer was designed to be 40\textdegree C, which is higher than a physiological temperature. The copolymers labeled with a rhodamine dye were injected into mice, and the delivery of the polymer to tumors with and without local heating above the LCST was monitored by fluorescence spectroscopy. The polymers were found accumulated on the heated solid tumor. This result suggested that thermal targeted drug delivery could be achieved by conjugation to thermally responsive polymers combined with local heating of tumors.

1.1.2 PolyNIPA gels and their volume phase transitions

Polymer gels consisting of weakly cross-linked polyNIPA swell and shrink in response to temperature,\textsuperscript{22} solvents\textsuperscript{23} and ions\textsuperscript{54,25} (Figure 1-1).\textsuperscript{26} PolyNIPA gels are swollen at room temperature and hold liquid water in their inside. However, at high
temperature (> 31°C), the gels shrink and squeeze water out. This volume change is reversible and similar to the gas-liquid transition of small molecules.\textsuperscript{27-29}

![Temperature, solvents, ions, pH, light, magnetic and electronic fields](image)

**Figure 1-1. Volume phase transition of polymer gels**

Metal ion adsorption by polyNIPA gels modified with carboxylate groups has been investigated.\textsuperscript{25,28} The carboxylate groups in the shrunken gel are close enough to capture a calcium ion by forming a multivalent (2:1) complex in water. Upon swelling, the carboxylate groups are located too far from each other to form such a complex, resulting in a release of ions.

---

* This volume change is regarded as a 1st-order phase transition because the gels exhibit discontinuous volume change (a volume is 1st derivation of Gibbs free energy, \( V = (\partial G / \partial P) \)). The shrunken and swollen states of the gels correspond to a liquid and gas state of small molecules, respectively. The example of a 1st-order discontinuous volume transition can be seen in liquid-gas transition of water: 18mL (1mol) of water becomes 22L of an ideal gas (vapor) at 100°C under 1atm pressure.

0 The molecular-level mechanism is the same in both the phase separation of linear polyNIPA and the volume change of gels, which is a dehydration/hydration from the polymer chains. However, the polymers have different conformational states in solutions and gels at high temperatures. In the case of phase separation from solution, the precipitate of the polymer is turbid because it is in an amorphous state in which the polymer chains are entangled intermolecularly, and it is not thermodynamically stable. In the case of gels, the swollen gel is transparent at low temperature and becomes opaque in the beginning of the shrinking at high temperature where the polymer chains aggregate and entangle temporary with other polymer chains, which is like in the case of the phase separation of linear polyNIPA. This opacity disappears over time, and the gel eventually becomes transparent. At this point, each polymer chain associates only with itself (coil-globule transition) and forms a thermodynamically stable phase at equilibrium. The polymer chains in the gel are cross-linked, so that strong entanglement of the polymer chains is prevented and they can therefore eventually shrink to exist in compact form.
1.2 Thesis overview

This thesis focuses on ion capture by polyNIPA gels and aggregation of fluorescent conjugated polymers (FCPs) by means of thermally induced phase separation of polyNIPA. PolyNIPA gels functionalized by N-vinylimidazole display altered ion adsorption behavior according to the gel swollen and shrunken states (Chapter 2). The thermal switching behavior of polyNIPA was employed to control the photo-physical properties of FCPs, and this function is being extended to biosensory applications in aqueous environments. FCPs possess unique electro-optical properties that enable ultra trace detection of analyte molecules. In the development of FCPs for biosensing, new non-ionic water-soluble FCPs have been synthesized. These polymers can be used to provide highly sensitive/specific ultra-trace detection that is immune to low specificity problems that often plague conjugated polyelectrolytes (Chapter 3). The controlled polymerization of NIPA was carried out using atom transfer polymerization (ATRP) (Chapter 4). By this method, polyNIPA was incorporated into water-soluble FCPs, and their thermally responsive properties were examined (Chapter 5). Finally, block copolymers of water-soluble conjugated polymers and polyNIPA have been synthesized.
via nitrooxide-mediated radical polymerization (NMRP), and the thermally induced fluorescence resonance energy transfer (FRET) has been demonstrated (Chapter 6).
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Chapter 2: Cu ion adsorption and catalysis by polymer gels

Abstract: Cu ion adsorption by formation of multivalent complexes with imidazole groups attached on a poly(N-isopropylacrylamide) (NIPA) network has been examined. The adsorption behavior was controlled by thermal swelling and shrinking of the polyNIPA gels. The catalytic activity of the gels for the decomposition of hydrogen peroxide was also dependent on the state of the gels. The swollen gel exhibited a higher activity than a monomeric Cu complex analogue and a Cu ion. The shrinking of the gel reduced the catalytic activity possibly due to restricted diffusion of H₂O₂ molecules into the gel.
2.1 Introduction

Polymer gels can capture and release an ion by multiple contact interaction and reversibly change the affinity for an ion through their thermally induced volume change.\textsuperscript{1,2,3,4,5} To have this property, the gels are modified with a minor component containing a receptor group such as a carboxylate group, which captures an ion by the formation of multivalent complexes. The major component forming a polymer network is responsible for gel shrinking and swelling in response to environmental changes such as temperature. In the swollen gel, the receptor groups are placed far from each other such that they cannot capture an ion. On the other hand, they can capture an ion in the shrunken state where the receptor groups are in proximity to each other enough to form a multivalent complex. Accordingly, a volume change of polymer gels leads to a reversible capture and release of an ion.

Ion adsorption by polymer gels through formation of electrostatic complexes has been examined.\textsuperscript{2,4} In this research, Cu (II) ion adsorption through a coordination bond by imidazole-functionalized polymer networks is investigated. N-isopropylacrylamide (NIPA) monomer was copolymerized with a minor component, N-vinylimidazole (VIm),
and a cross-linker, \textit{N,N}-methylene bis-acrylamide (BIS). In our design, the major component, polyNIPA, controls the thermal swelling and shrinking of the gel.

\[ \begin{align*}
N\text{-Isopropylacrylamide (NiPA)} & \quad N\text{-Vinyldazole (Vim)} & \quad N,N\text{-methylenebisacrylamide (BIS)}
\end{align*} \]

It is known that imidazole (Im) forms a multivalent complex with Cu (II) ion in water at the equilibrium;

\[
\begin{align*}
Cu + Im & \overset{b_1}{\longrightarrow} Culm_1 \\
Culm_1 + Im & \overset{b_2}{\longrightarrow} Culm_2 \\
Culm_2 + Im & \overset{b_3}{\longrightarrow} Culm_3 \\
Culm_3 + Im & \overset{b_4}{\longrightarrow} Culm_4 \\
\end{align*}
\]

The adsorption isotherms were analyzed using the Langmuir isotherm. The Langmuir adsorption isotherm for ion adsorption by receptor groups in a polymer gel (Figure 2-1,b) is given by

\[
A = \frac{SKC_{eq}}{1 + KC_{eq}} \quad (1)
\]

where \( C_{eq} \) is the final equilibrium concentration of ions in the solution. \( A \) is the amount of the ions adsorbed by unit volume of the gel in the shrunken state. \( S \) and \( K \) are the number of binding sites and the apparent binding constant of one binding site, respectively (The derivation of the Langmuir isotherm for adsorption of an ideal gas on a solid surface is discussed in Appendix, wherein \( P \) and \( S_{occ} \) are replaced by \( C_{eq} \), and \( A \) to give Equation 1). Base on Equation 1, the saturation value of the isotherm in Figure 2-1
(a), corresponds to $S$. The equation of the initial tangent is $A = SKC_{eq}$, so that the initial slope of the isotherm is given by $SK$. The $C_{eq}$ at the intersection of the initial tangent and the line of $A = S$ gives $1/K$.

![Graph showing the Langmuir isotherm](image)

Figure 2-1. (a) The Langmuir adsorption isotherm plotted based on Equation (1). (b) Ion adsorption by a polymer gel. $A$ and $C_{eq}$ are the ion concentrations in the gel and in the solution.

In addition, Cu-Imidazole complexes were found to catalyze the decomposition of $H_2O_2$:

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad (2)$$

The catalytic activity of the gel is also examined in correlation with the thermal volume change of the gel.

2.2 Experimental results and discussion

2.2.1 Cu(II) ion adsorption

Figure 2-2 shows adsorption isotherms of Cu (II) ions by imidazole-functionalized polyNIPA gels. The gel was swollen at room temperature and shrunken at 60°C. The adsorption data was curve-fitted by the Langmuir isotherm equation (1). The gel containing 96mM $N$-vinylimidazole (VIm) showed almost the same isotherms in the swollen and shrunken state (Figure 2-2, a). On the other hand, the gel containing 24mM
VIm behaved differently in the two isotherms. The swollen gel adsorbed larger amount of Cu ions than the shrunken gel although the initial slopes were the same. These results indicate:

1. When the concentration of VIm in the gel ([VIm]) is high ([VIm] = 96mM, Figure 2-2 (a)), the distance among VIm groups is small enough to form a complex with a Cu ion even in the swollen state, resulting no difference in the adsorption of Cu ions by the swollen and shrunken gels.

2. The saturation value of the isotherm (Figure 2-2 (a)) $S$, which gives the number of binding sites in the gel, is about $[\text{VIm}]/4 (= 24\text{mM})$, which suggests that the binding site consists of four VIm groups (formation of CuVIm$_4$).

3. In the case of $[\text{VIm}] = 24\text{mM}$ (Figure 2-2 (b)), the saturation value of the shrunken gel $S$ is 4.14mM, which comparable with $[\text{VIm}]/4 (= 6\text{mM})$. The $S$ of the swollen gel is 10.67mM, which is close to $[\text{VIm}]/2 (= 12\text{mM})$. This indicates the formation of CuVIm$_4$ and CuVIm$_2$ for the shrunken and swollen gels, respectively.

4. The swollen gel adsorbed more Cu ions than the shrunken gel at high Cu ion concentrations (Figure 2-2, b, $[\text{VIm}] = 24\text{mM}$). The swollen gel has more available binding sites than the shrunken gel.
Figure 2-2. Adsorption isotherms of Cu (II) ions by the swollen gel at room temperature and the shrunken gel at 60°C. A and C_{eq} are the concentration of CuCl₂ in a unit volumes of a shrunken gel and in the solution at equilibrium, respectively. (a) [VIm] in gel = 96mM, (b) 24mM. The data were curve-fitted by the Langmuir equation (solid line and broken line). The solution contained NaCl (100mM). The concentrations of NIPA and BIS upon synthesis are 6M and 40mM, respectively.

S, K and the overall affinity SK were obtained from adsorption isotherms using gels that contain different [VIm] (128-16mM) (Figure 2-3). The S of the shrunken gels is proportional to [VIm] in the gel and corresponds to [VIm]/4, which suggests the formation of CuVIm₄. The S of the swollen gel is higher than that of the shrunken gel at the low [VIm]. This indicates that the number of VIm groups in one complex in the swollen gel decreased in low [VIm] since the swelling of the gel prevents the formation of CuVIm₄.

In Figure 2-3, SK is proportional to [VIm]² for both the swollen and shrunken gels. This result suggests that two VIm groups formed one complex with one Cu ion. Since VIm groups are randomly distributed in the polymer network, the probability for one VIm to find another VIm in its vicinity is proportional to its concentration. This leads an apparent binding constant K, which is proportional to [VIm]. The number of
binding sites $S$ is also proportional to $[\text{VIm}]$. Therefore, $SK \propto [\text{VIm}]^2$. In other words, $SK$ is an initial slope of adsorption isotherms (Figure 2-1, a) and represents the probability that two VIm groups come into proximity in any given location, which is proportional to $[\text{VIm}][\text{VIm}] = [\text{VIm}]^2$. This is found to have an analogy to an initial rate method for determination of a reaction order of chemical reactions. The slope of a log-log plot of initial rates and initial reactant concentrations gives the order of the reaction. For example, suppose we consider about a dimerization reaction $(\text{reactant} + \text{reactant} \rightarrow \text{dimer})$, where one reactant has to find another reactant to give a dimer. Therefore, the reaction rate of this dimerization is proportional to $[\text{reactant}][\text{reactant}] = [\text{reactant}]^2$.

From this analogy, one receptor finds another receptor to form a complex such that an initial slope of an adsorption isotherm $SK$ is proportional to $[\text{receptor}][\text{receptor}] = [\text{receptor}]^2$. The results from $SK$ suggest that formation of CuVIm$_2$ is responsible for Cu ion adsorption by the shrunken gel, whereas the $S$ values indicate the formation of CuVIm$_4$. Moreover, no difference between $SK$ values of the swollen and shrunken gels was observed although the $S$ showed differences.

These results from $S$ and $SK$ indicate that the dominant complex is CuVIm$_2$ for initial adsorption and CuVIm$_4$ for high Cu concentrations close to the saturation of the binding sites. In addition, the fact that there is no difference in $SK$ values of the swollen and shrunken gels means that the formation of CuVIm$_2$ is irrelevant with the distance between VIm groups. This could be due to that CuVIm$_2$ was formed by two VIm groups in vicinity or that are intrachain rather than those randomly distributed VIm groups. Such intrachain complex can be stable and dominant in the adsorption due to the lack of the stretching of the polymer chains.
The ion adsorption by gels using electrostatic complexation such as (1) MAFTAC-pyranine\textsuperscript{2} and (2) methacrylic acid-calcium ion\textsuperscript{4} has been explained by the analysis using $S$ and $SK$.

(1) Oya \textit{et al.} reported adsorption of anions (pyranine-3 and pyranine-4) by polyNIPA modified with a cationic monomer, methacrylamide-propyltrimethylammonium chloride (MAFTAC), by the formation of electrostatic complexes.\textsuperscript{2} The pyranine molecules were adsorbed by the shrunken gels and released from the swollen gels. The initial slope ($SK$) of the adsorption isotherms, which was referred to as “affinity” in the report,\textsuperscript{2} was analyzed with changing MAFTAC concentrations, [MAFTAC], in the gels. The affinity showed a power law, and it is proportional to [MAFTAC]\textsuperscript{3} for pyranine-3 and [MAFTAC]\textsuperscript{4} for pyranine-4 in the adsorption by the shrunken gels, which indicates multiple-point adsorption of pyranine.
molecules by the gels. In the case of the swollen gels, the affinity $\propto [\text{MAPTAC}]$, which means that one MAPTAC binds one pyranine molecule.

(2) Alvarez-Lorenzo et al. demonstrated multivalent calcium ion binding by methacrylic acid (MAA) attached on polyNIPA networks. The isotherm was fitted by the Langmuir isotherm equation. Their experiment showed that $SK \propto [\text{MAA}]^2$, which indicates that carboxylate groups form a 2:1 complex with calcium ions in the gel. The $S$ corresponds to [MAA]/2, which also suggests the formation of such a complex.

Based on these results from ion adsorption through electrostatic complexation, $SK$ of the Cu adsorption through coordination boning studied here was initially expected to show a power law $SK \propto [\text{VIm}]^4$ for the formation of CuVIm$_4$. However, this experiment showed $SK \propto [\text{VIm}]^2$. In addition to the intrachain complexation mentioned above, other possible effects are listed here.

(1) The complexation of Cu ions through a coordination bond may have different nature from electrostatic complexation.

(2) The positive charges of Cu-complexes on the polymer chains make the polymer network expand, and complexation of multiple VIm groups produces cross-linking of polymer chains.
(3) The hydrophobic environment of the shrunken gels also could affect the adsorption behavior. The high density of polymer chains and the hydrophobicity of the shrunken gels may prohibit diffusion of Cu ions and their complexation with VIm because the formation of ionic complexes is energetically unfavorable in hydrophobic environment.

(4) The Cu ions bound on the gel surface could create an ionic layer, which further discourages diffusion of ions into the gel. This layer effect has been observed in uptake of surfactants by polyNIPA gels.⁹

### 2.2.2 Cross-linker concentration dependence

The cross-linker concentration ([BIS]) dependence of Cu adsorption was examined. Log-log plots of $S$, $K$ and $SK$ are shown in Figure 3, 5 and 6 for [BIS] = 40, 10 and 100mM, respectively. It is expected that increased [BIS] prevents the formation of multivalent Cu-VIm complexes because the cross-linkers limit the diffusion of polymer chains. The shrunken gel, however, showed $S = [\text{VIm}]/4$, indicating formation of CuVIm₄. The cross-linker did not prevent the formation of CuVIm₄ in this [BIS] region. The complexation energy of CuVIm₄ is still dominant over the elastic energy of polymer chains caused by the cross-linker. In the case of the swollen gels, at low [BIS] (10 and 40mM), the $S$ is larger than the shrunken gel at low [VIm]. A significant difference in the $S$ is not found at [BIS] = 100mM. In general, a volume of a swollen gel increases with decreasing a cross-linker concentration. The large swelling of the polymer network results in large distances among ligand groups that prevent the formation of multivalent Cu-VIm complexes. As a result, the number of the binding site increases

27
with decreasing of [BIS] in the swollen gels. The $SK$ values are irrelevant with a change of [BIS], and $SK \approx [VIm]^2$, which suggests the formation of VIm$_2$ is responsible for the initial Cu adsorption in this [BIS] region (10-100mM).

Figure 2-4. Log-log plots of $S$, $K$ and $SK$ as a function of VIm/4 for gels containing 10mM BIS. The solution contained NaCl (100mM). The concentration of NIPA was 6M in gel synthesis.

Figure 2-5. Log-log plots of $S$, $K$ and $SK$ as a function of VIm/4 for gels containing 100mM BIS. The solution contained NaCl (100mM). The concentration of NIPA was 6M in gel synthesis.
2.2.3 Buffer effect

A sodium acetate buffer solution is expected to have two effects on Cu ion adsorption by the gels:

(1) Competition: Acetic acid is capable of formation of a complex with Cu ions, which competes with Cu-VIm complexation, resulting in a decrease of the affinity for Cu ions, i.e. a decrease in $SK$ values.

(2) Protonation of VIm: VIm is expected to be partially protonated in this buffer solution pH = 5.6 because pKa of its conjugated acid of imidazole is 7.\(^\text{10}\) The protonation of VIm will compete with Cu ion, resulting in a decrease of the affinity for Cu ions.

The $S$ of the shrunken gel corresponds to $[\text{VIm}]/4$, which indicates formation of CuVIm\(_4\). The $SK$ showed a power law, $SK \propto [\text{VIm}]^2$. The values, however, are 10 orders of magnitude smaller than that of the NaCl system shown in Figure 2-3. These results suggest that the affinity for Cu ions was reduced by using buffer solution without changing the binding scheme from that in NaCl solution (Figure 2-3).
2.2.4 Catalytic decomposition of $\text{H}_2\text{O}_2$

Multivalent Cu-Im complexes are found to catalyze the decomposition of hydrogen peroxide.\textsuperscript{7} Possible reaction mechanisms have been proposed in terms of the coordination of $\text{H}_2\text{O}_2$ to Cu and the formation of $\text{IIOO}^-$.\textsuperscript{7} Cu-VIm complexes in the gel are also expected to catalyze the decomposition of $\text{H}_2\text{O}_2$, and the polymer density change may affect the catalytic activity. The discussion about the reaction mechanism and the reaction kinetics, however, is beyond the scope of this research. Here, a catalytic activity depending on swelling and shrinking of polymer gels is focused on. PolyNIPA gels containing 400mM VIm were soaked in 1mM Cu(NO$_3$)$_2$ solution (sodium acetate buffer, 10mM, pH5.6). The gel shrinks above 35$^\circ$C (Figure 2-7, a). After addition of concentrated $\text{H}_2\text{O}_2$ solution, the volume of evolved oxygen gas from the reaction solution was measured with time (Figure 2-8). At 25 and 35$^\circ$C, where the gels were swollen, the reaction rate in the presence of the gel is larger than other solutions containing only a Cu ion or a monomeric analogue, Cu-$N$-methylimidazole (MIm) complex. The reaction
conversion reached to about 7% and 13% after 60 minutes at 25 and 35°C, respectively. On the other hand, the reaction rate of the shrunken gel at 40°C has no difference from the solutions containing Cu or Cu-MIm complex. The initial rates of the reaction curves are plotted as a function of temperature in Figure 2-7, b. The swollen gel exhibited larger reaction rates than other solutions containing Cu or Cu-MIm complex, indicating that the formation of Cu complexes is enhanced in the gel. However, the initial rate of the gel showed a maximum around 30°C where the gel starts to shrink, whereas the solution of Cu ion or Cu-MIm showed a monotonic increase of the reaction rates with increasing temperature. The temperature dependence of the gel catalysis is possibly related with diffusion of H₂O₂ into the gel. The shrunken gels have a high density of polymer chains and hydrophobic environment, which restricts diffusion of H₂O₂ molecules into the gel, resulting in a decrease of the reaction rates at high temperatures.

\[ \text{N-Methylimidazole (MIm)} \]
Figure 2-7. (a) Relative diameter of gels to the initial diameter (250μm) upon synthesis. (b) Initial reaction rates of the decomposition of H₂O₂. See the caption of Figure 2-8 for the reaction condition. N-Methylimidazole (MIm) was used as a monomeric analogue.

Figure 2-8. The decomposition of H₂O₂ catalyzed by polymer gels. The concentration of VIm in the gel was 400mM and 20μL of the gel was used. The reaction was carried out in 20 mL of 10mM sodium acetate buffer, pH 5.2 in the presence of 1mM Cu(NO₃)₂. The same amount of N-Methylimidazole (MIm) as VIm in the gels was added to the Cu solution for comparison ([MIm] = 0.4mM). Initial H₂O₂ concentration was 8.9mM.
2.3 Conclusion

Cu ion adsorption by the formation of multivalent complexes through a coordination bond with imidazole groups attached on a polyNIPA network has been examined. The adsorption behavior was altered by the thermal swelling and shrinking of the gel. The adsorption isotherm was analyzed based on the Langmuir adsorption equation. These results from $S$ and $SK$ indicate that the dominant complex is $\text{CuVIIm}_2$ for initial adsorption and $\text{CuVIIm}_4$ for high Cu concentrations close to the saturation of the binding sites, which is different from the previous results using electrostatic complexes for ion adsorption. The catalytic activity of the gels for the decomposition of $\text{H}_2\text{O}_2$ was also investigated. The swollen gel exhibited higher activity than a monomeric Cu complex and a Cu ion. The shrinking of the gel reduced the catalytic activity possibly due to restricted diffusion limit of $\text{H}_2\text{O}_2$ molecules into the gel.
2.4 Experimental procedures

2.4.1 Gel preparation

The polymer gels were prepared by free radical polymerization of N-isopropylacrylamide (NIPA), methylenebis(acrylamide) (BIS) and N-vinylimidazole (VIm) using 2,2'-azobis(isobutyronitrile) (AIBN). The monomer solution was prepared in DMSO. The monomer concentrations are [NIPA] = 6M, [BIS] = 10-100 mM, [VIm] = 128-8 mM. After addition of AIBN (5mM), the solution was transferred to a glass test tube in which small glass pipettes (inner diameter 250 μm) are placed. The test tube was heated at 60°C overnight, and the pipettes were taken out from the test tube. The cylindrical gels were taken out by carefully breaking the pipettes and washed with water to remove unreacted monomers and solvent. The gels were dried under vacuum for several days.

2.4.2 Adsorption of Cu ions by the gels and determination of Cu concentrations

Several pieces of the dried gel (c.a. 2mg) were placed in a 2mL plastic microcentrifuge tube with a screw cap into which CuCl₂ aqueous solution (0.02-0.16 mM, 1.5mL) containing 100 mM NaCl or 100 mM sodium acetate buffer solution was added. The vials were left at room temperature allowing the gels to swell for several days. The concentration of Cu ions was determined by a colorimetric method using 4-(2-pyridylazo)-resorcinol (PAR).\textsuperscript{11}
The solution (0.2mL) was taken from the vial and diluted by a borax buffer solution (pH 8.3, 1mL) containing PAR. The PAR molecule forms a chelate complex with a Cu (II) ion and gives UV-vis absorption maximum at 500 nm. The amount of Cu ions in the sample solution was determined by the absorbance at 500nm from the molar absorption coefficient $\varepsilon$ of the PAR-Cu complex. The $\varepsilon$ was obtained from a plot of the absorbance at $\lambda = 500$nm and Cu concentrations, and the typical $\varepsilon$ value is $5.954 \times 10^4$ absorbance/M for 100mM NaCl sample solution/borax buffer. The amount of Cu ions absorbed by the gel was calculated by the difference between the initial and final concentrations of Cu ions in the solutions. The other set of the samples was also left at room temperature for several days and then heated at 60$^\circ$C in a glass beads bath for 5-7 days. The amount of Cu ions in the shrunken gel was determined in the same way as that for the swollen gels. The data was analyzed using the Langmuir isotherm equation:

$$A = \frac{SKC_{eq}}{1 + KC_{eq}}$$

(1)

where $C_{eq}$ is equilibrium concentration of the Cu ion in the solution. $A$ is the amount of Cu ions per unit volume of the gel in the shrunken state. $S$ and $K$ are the number of binding sites and apparent binding constant, respectively. The $S$ and $K$ values were estimated by the direct fitting on the isotherms.

2.4.3 Catalysis

PolyNIPA gels containing 400mM of V1m were used for catalysis of the decomposition of $\text{H}_2\text{O}_2$. The cylindrical gel (20µL) and a magnetic stir bar were placed in a 25mL round bottom flask into which 20mL of 1mM $\text{Cu(NO}_3\text{)}_2$ sodium acetate buffer (10mM, pH 5.2) was added. The flask was placed in a water bath. The temperature of
the bath was controlled by a re-circulating chiller. The gel samples were heated at the desired temperature one day before the measurements. After concentrated H₂O₂ solution was injected into the solution, the flask was connected to a pipette filled with water to collect oxygen gas. The initial concentration of H₂O₂ was 8.9mM. The volume of produced oxygen gas was measured at room temperature.
2.5 Appendix: Langmuir isotherm for gas-solid surface adsorption

The Langmuir isotherm for adsorption of an ideal gas by receptor sites on a solid surface is introduced using simple kinetic theory. We define the following parameters:

(1) At equilibrium, the number of the occupied sites and unoccupied sites is \(S_{oc}\) and \(S_{unoc}\). The total number of the site \(S\) is given by

\[
S = S_{oc} + S_{unoc}
\]  
(3)

(2) \(\theta\) is defined by

\[
\theta = \frac{S_{oc}}{S}
\]  
(4)

Thereby,

\[
1 - \theta = \frac{S_{unoc}}{S}
\]  
(5)

The assumptions are:

(1) One receptor site binds only one particle.

(2) The binding constant of each site is not dependent on \(\theta\).

(3) There are no interactions among the bound particles.

![Figure 2.9](image-url)  
**Figure 2.9.** Adsorption of a gas by a receptor site on a surface.

The dissociation rate of the particle from the site is proportional to \(\theta\) so that
Dissociation rate = \( k_d \theta \)  \hspace{1cm} (6)

where \( k_d \) is a rate constant.

The adsorption rate is proportional to \((1-\theta)\) and a gas pressure \( P \) because the collision frequency of gas molecules on the surface is proportional to \( P \).

\[
\text{Adsorption rate} = k_a P(1 - \theta) \hspace{1cm} (7)
\]

where \( k_a \) is a rate constant.

At equilibrium, the dissociation rate equals the adsorption rate. Therefore,

\[
k_d \theta = k_a P (1 - \theta) \quad \text{or} \quad \theta = \frac{K P}{1 + KP} \hspace{1cm} (8)
\]

where \( K = k_f / k_d \).

From the equation (8) and definition (4),

\[
S_{\text{occ}} = \frac{SKP}{1 + KP} \hspace{1cm} (9)
\]

The equation (9) can be written as

\[
\frac{P}{S_{\text{occ}}} = \frac{1}{SK} + \frac{P}{S} \hspace{1cm} (10)
\]

Figure 2-10. The Langmuir adsorption isotherm plotted based on (a) eq. 9. (b) and eq. 10.
2.6 References

10 "*Handbook of Chemistry and Physics*", D. R. Lide, ed. 78th, CRC press
Chapter 3: Non-ionic water-soluble conjugated polymers

Portions of this chapter are adapted from:


Abstract: Hydrophilic conjugated polymers have been synthesized that will be a platform for biosensory materials. High water-solubility has been achieved by dendritic structures with numerous hydroxyl and amide groups on the side chains.
3.1 Introduction

Conjugated polymeric materials\(^*\) are of great interest because of the high sensitivity of their optical and conducting properties in the presence of analytes\(^{1,2}\). In particular, water-soluble conjugated polymers offer powerful new methods for the trace detection of analytes in an aqueous environment. However, applications of water soluble conjugated polymers to aqueous sensing have been limited as a result of the challenges associated with strong interactions between hydrophobic backbones and aromatic \(\pi-\pi\) stacking that severely restrict their water solubility. The conventional approach to water-soluble conjugated polymers employs ionic groups such as sulfonic\(^3\), carboxylic\(^4\) or ammonium groups,\(^5\) usually in the side chains. These groups give strong enthalpic interactions with water, which keeps the polymers in solution. Moreover, the electrostatic repulsions between the polymer chains of like charge also promote their water solubility. Ionic conjugated polymers or conjugated polyelectrolytes have been applied in biosensor schemes.\(^5,6,7,8,10\) Taking an advantage of the fact that conjugated polyelectrolytes have the ability to form electrostatic complexes with ionic molecules, new detection schemes have been proposed and examined.

Chen \textit{et al.}\(^8\) have recently made use of amplification principles demonstrated by the Swager’s group\(^2\) and have reported that fluorescence of a water-soluble anionic poly(phenylene-vinylene) (PPV) derivative with sulfonate group on the side chain (MPS-

\(^*\) We have focused on conjugated polymers with rigid rod-like structures consisting of aromatic groups connected directly or with double or triple bonds, where \(\pi\)-electrons are delocalized along the polymer chain.

\begin{align*}
\text{Poly(phenylene-ethynylene)} & \quad \text{Poly(phenylene-vinylene)} & \quad \text{Poly(phenylene)} \\
\end{align*}
PPV) was quenched efficiently by methylviologen (MV$^{2+}$) as shown in earlier studies of the Swager's group$^9$. The strong fluorescence quenching of MPS-PPV results from their formation of an electrostatic complex of the anionic polymer and the cationic quencher. In this scheme, the fluorescence of MPS-PPV is quenched by biotinylated MV$^{3+}$, B-MV, which binds electrostatically to the polymer. The fluorescence of the polymer was restored upon removal of the quencher through avidin binding. Also building upon these amplification principles, MPS-PPV has been shown to be quenched electron transfer protein, cytochrome c (cyt c), as reported by Fan et al.$^6$ and DNA detection using a cationic conjugated polymer (CCP) was reported by Gaylord et al.$^{10}$ This latter scheme makes use of the fluorescence resonance energy transfer (FRET)$^7$ method previously reported by McQuade et al.$^{11}$ A fluorescein-labeled neutral peptide nucleic acid (PNA-C$^*$) that has the complementary sequence of a target DNA was paired with DNA/CCP hydrides by nucleic acid base pair matching. In this process, the target DNA served as a specific interaction to connect a fluorescent probe (PNA-C$^*$) and CCP. This complexation brings the fluorescein in the vicinity of CCP and thus results in signal amplification from the fluorescein by FRET.

\[ \text{Methylviologen (MV}^{2+}\text{)} \quad \text{MPS-PPV} \quad \text{CCP} \quad \text{Carboxylate-PPE} \]

* FRET is a distance-dependent through space dipole interaction of chromophores (a donor and an acceptor). The energy of the donor is transferred to the acceptor without emission when the distance separating them is in the range of 50-100 Å. When the donor excited, the energy of the excited state of the donor is transferred to the acceptor through space without donor emission, and the emission from the acceptor is observed.

$^6$ The acceptor can receive energy effectively from multiple polymer segments, which have been transported over large distances; so FRET produces an amplified emissive signal from the acceptor, which leads to light-harvesting.
In spite of these laboratory demonstrations, ionic conjugated polymers have severe limitations for use in biosensor schemes for the following two reasons. Firstly, the solution conditions (pH, ionic strength, temperature) have to be adjusted to prevent polymer aggregation. Secondly, electrostatic interactions between ionic polymers and biomolecules, such as proteins and DNA, are non-specific and therefore will reduce specificity for target molecules. Considering these demerits of ionic sensory polymers, we have chosen to develop nonionic (neutral) water-soluble conjugated polymers as a platform for high specificity biosensory polymers.

3.2 Results and discussion

3.2.1 Hydrophilic conjugated polymers

To achieve high water-solubility without ionic groups, poly(phenylene-ethynylene) (PPE)* derivatives with non-ionic hydrophilic groups such as hydroxyl and amide groups in the side chains have been investigated. The extremely hydrophobic nature of the PPE backbone made it necessary to place a high density of hydroxyl groups proximate to the polymer backbone and thereby shield it from water. Based upon this design principle, a number of nonionic polymers have been synthesized that are summarized in Table 3-1. Finally, completely water-soluble PPE (Polymer A, No. 11,

* We have focus on synthesis of PPE derivatives because the polymerization conditions are relatively tolerant to the side chain functionalities of the polymers.
Run #1, Table 3-1) with hydroxyl and amide groups surrounding polymer chain was obtained and used as a platform for further investigations.

**Table 3-1. Hydrophilic poly(phenylene-ethynylene)s and polymerization conditions**

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical structure R</th>
<th>R'</th>
<th>Solvent</th>
<th>Molecular weight (GPC eluent: DMF)</th>
<th>Properties</th>
</tr>
</thead>
</table>
| 1   | ![Structure](image)  |    | Morpholine      | Run #1   
Mn = 16,078  
Mw = 31,899  
PDI = 1.98  
n = 16  
0.1 mg/mL after 60°C overnight |
|     |                      |    |                 | Run #2   
Mn = 12,487  
Mw = 24,122  
PDI = 1.93  
n = 15 (bimodal) |
| 2   | ![Structure](image)  |    | DMF/DIPA        | crashed out during polym.                             | No polym.                  |
| 3   | ![Structure](image)  |    | NMP/Et₃N        | No polym.                                             |                          |
| 4   | ![Structure](image)  |    | N-methyl-morpholine/water | Monomers insoluble No polym. |                          |
| 5   | ![Structure](image)  |    | Et₃N            | Monomers insoluble No polym.                          |                          |
| 6   | ![Structure](image)  |    | Hunig’s base    | Monomers insoluble No polym.                          |                          |
| 7   | ![Structure](image)  |    | Morpholine      | Mn = 11,364  
Mw = 21,170  
PDI = 1.86  
n = 13  
sl. s.            |
| 8   | ![Structure](image)  |    | Morpholine      | Mn = 40,314  
Mw = 323,69  
PDI =  
n = 33 (bimodal) |
<p>| 9   | <img src="image" alt="Structure" />  |    | DMF/DIPA        | i. (water)                                            |                          |
| 10  | <img src="image" alt="Structure" />  |    | Morpholine      | sl.s. (water) Fibril solid                           |                          |</p>
<table>
<thead>
<tr>
<th>Run #1</th>
<th></th>
<th></th>
<th>(M_n = 31,817)</th>
<th>(M_w = 45,115)</th>
<th>(PDI = 1.41)</th>
<th>(n = 24) (before dialysis)</th>
<th>s. (water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run #2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dialysis with (\text{NH}_4\text{OH}) against water. s. (water) precip. in polym.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>Morpholine (orange precip.)</td>
<td>-</td>
<td>-</td>
<td>s. (water)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>-</td>
<td>s. (water)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>Morpholine (precipit.)</td>
<td>-</td>
<td>-</td>
<td>s. (water)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>-</td>
<td>Oligomer, coupled with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>-</td>
<td>w/ an end-cap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>-</td>
<td>w/ an end-cap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

s: soluble; sl: slightly; i: insoluble

3.2.2 Synthesis of hydrophilic monomers

Polymer A (No. 11 Run #1, Table 3-1) was synthesized by a Sonogashira cross-coupling of diethynylene benzene derivatives 6 and diiodobenzene 9 prepared according to Schemes 3-1 and 3-2 respectively. As shown in Scheme 3-1, diiodohydroquione 1 was
reacted with ethyl bromoacetate to give ester 2, which after reaction with diethanol amine was treated with triisopropylsilyl chloride in THF to give organic soluble 3 that is easily purified by re-crystallization from CH$_2$Cl$_2$/methanol. Compound 5 is obtained from 3 by standard Sonogashira cross-coupling procedures. The TIPS groups were removed by treatment with tributylammonium fluoride (TBAF) in THF to give the compound 6 which precipitates from MeOH/CH$_2$Cl$_2$. Diiodobenzene derivative 4 was made by cleavage of TIPS groups from 3 in the same procedure for 6 or alternatively from the t-butyldimethylsilyl protected variant.
The most hydrophilic monomer 9 was prepared in three steps from 2 (Scheme 3-2). The ester group of 2 is hydrolyzed, converted to the acid chloride, and reacted with diethyl iminodiacetate to give 8. The target compound 9, was afforded by adaptation of Newkome’s synthesis of arborols.\textsuperscript{12} Compound 8 is treated with tris(hydroxymethyl)aminomethane (Tris) in DMSO in presence of K\textsubscript{2}CO\textsubscript{3} at room temperature.\textsuperscript{12,13} After workup, the oily residue was rinsed with CH\textsubscript{2}Cl\textsubscript{2} to give 9 as a precipitate. In this synthetic approach, 9 was obtained without protection of the hydroxyl group.

* The proposed reaction mechanism is the following:\textsuperscript{12} First, the alkoxide of Tris was formed by a base (K\textsubscript{2}CO\textsubscript{3}). Then, this alkoxide reacts with the triester via transesterification. Finally, an amide is formed by an intramolecular rearrangement.
group in contrast to the synthesis of 6. This monomer is completely soluble in DMF, DMSO, and water.

Scheme 3-2

![Scheme 3-2](image)

The other attempts to make highly hydroxylated monomers were less successful. Initially, amide formation of the triester molecules (12, 14, 17) with Tris was attempted according to the Newkome's procedure\textsuperscript{12,13,14}, however the desired compounds were not afforded (Scheme 3-3). Considering the proposed reaction mechanism (see the footnote in section 3.2.2.),\textsuperscript{12} this reaction might be accompanied by de-carboxylation, thereby giving intermediates that resist amide formation and result in carboxylated products instead of amide compounds.\textsuperscript{12,14}
3.2.3 Polymerization

Monomers 6 and 9 (54mM) were copolymerized by a Sonogashira cross-coupling reaction in the presence of 5% (PPh₃)₄Pd and 5% CuI in morpholine at 50°C overnight. The polymer was precipitated in ethyl acetate and dried under vacuum. The obtained Polymer A is readily soluble in water, dissolving completely in all proportions.
instantaneously without heating. Gel permeation chromatography analysis (GPC) (elucent: DMF, PMMA standards) indicated that the $M_n$ and $M_w$ of the obtained Polymer A are 45,000 and 32,000, respectively. A homopolymer, Polymer B, was also synthesized by coupling 4 and 6 in the same polymerization procedure ($M_n = 12,487$, $M_w = 24,122$). However this polymer is only slightly soluble (est. 0.1 mg/ml) in water after heating at 60°C overnight.

Another approach for the synthesis of hydrophilic polymers was using TIPS-protected precursor polymers and their deprotection. TIPS-protected polymers were synthesized in toluene/DIPA (Table 3-2). However, the deprotection of TIPS using TBAF in THF/MeOH was not completed because the deprotection dramatically changed the solubility of the polymers, and thereby the partially deprotected polymers precipitated out from the solvent.

<table>
<thead>
<tr>
<th>Table 3-2. Polymers with protected hydroxyl groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td><img src="image" alt="Reactions" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical structure</th>
<th>Solvent</th>
<th>Molecular weight (GPC eluent: THF)</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Chemical structure" /></td>
<td><img src="image" alt="Solvent" /></td>
<td>Run #1 $M_n = 34,430$ $M_w = 68,345$ $PDI = 1.99$ $n = 16.4$</td>
<td>s. (THF, CH$_2$Cl$_2$, DMF) i. (water)</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Chemical structure" /></td>
<td><img src="image" alt="Solvent" /></td>
<td>Run #2 $M_n = 58,300$ $M_w = 205,000$ $PDI = 3.53$ $n = 23$</td>
<td>s (THF, CH$_2$Cl$_2$, DMF) i. (water)</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Chemical structure" /></td>
<td><img src="image" alt="Solvent" /></td>
<td>oligomer</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Chemical structure" /></td>
<td><img src="image" alt="Solvent" /></td>
<td>$M_n = 45,800$ $M_w = 81,500$ $PDI = 1.78$ $n = 2.2$</td>
<td>-</td>
</tr>
</tbody>
</table>

s: soluble; sl: slightly; i: insoluble
3.2.4 Optical properties of Polymer A

UV-vis and fluorescence spectra of aqueous and DMF solutions of Polymer A are shown in Figure 3-1. The aqueous solution prepared by dissolving Polymer A in water at room temperature showed small peaks in the higher wavelength region of the absorption and fluorescence. These features disappeared after heating at 60°C overnight. Aggregation of conjugated polymers generally produces such new low energy absorption and emission features. Therefore, the spectral change indicates that Polymer A is initially slightly aggregated, but it dissociates after heating. Quantum yields of Polymer A were 0.07 in water and 0.27 in DMF determined with quinine sulfate as a standard. The origin of the reduced quantum yield in water is unclear. It is still possible that the strong hydrogen bonding tendency of the side chains produces an unfavorable organization of the polymers. Although aggregation is not apparent from the spectra after heating, a very weak aggregation cannot be ruled out. Newkome reported aggregation and gelation in water of arborols, which consist of two hydrophilic Tris-based dendron spheres connected with a lipophilic bridge such as an alkyl chain or phenyl groups.12,14
3.2.5 Nonionic hydrophilic thiophene-containing polymers

To obtain water-soluble poly(arylene-ethynylene)s (PAEs) with different band gaps, thiophene-containing polymers (PTs) were synthesized. Thiophene groups in PAE polymer backbones generally create a smaller energy band gap and give absorption and emission peaks at longer wavelength compared with PPE type polymers. The monomers 20 and 23 were synthesized by adapting Newkome’s method\textsuperscript{12} (Scheme 3-4). The synthesized PTs are listed in Table 3-3. The PTs are not fluorescent in water, possibly due to the strong association of polymer chains caused by the highly hydrophobic polymer backbone. The side chains are probably not hydrophilic enough to solubilize and isolate each polymer chain by hydration. UV-vis absorption and fluorescence spectra of the selected polymers, No. 4 and 7 (Table 3-3) in DMF or water were shown in Figure 3-2 and 3-3, respectively.
Scheme 3-4

Table 3-3. Hydrophilic thiophene-containing polymers

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical structure</th>
<th>Solvent</th>
<th>Molecular weight (GPC eluent: DMF)</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>DMF/DIPA</td>
<td>-</td>
<td>s. (DMF) i. (water)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>i. (water)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>i. (water)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>i. (water)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Toluene/DIPA</td>
<td>oligomer</td>
<td>s (THF, CHCl₃)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>s. (water), no fluorescence</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Morpholine</td>
<td>-</td>
<td>sl. s. (water), weak fluorescence</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>K₂CO₃, MeOH/Morpholine end-cap</td>
<td>-</td>
<td>Partially s. In situ deprotection i (DMF)</td>
</tr>
</tbody>
</table>
3.3 Conclusion

New non-ionic hydrophilic PPEs and thiophene-containing conjugated polymers have been synthesized that have a number of hydroxyl and amide groups on the side
chains. Through the synthesis of a number of non-ionic hydrophilic polymers, it can be concluded that the side chains should have hydrophilic dendritic structure with Tris that can surround the hydrophobic backbone and shield it from water to achieve a high water-solubility. However, because of hydrogen bonding nature of the side chains, the polymer might be aggregated even though no spectroscopic evidence in the spectra was observed. To prevent this unfavorable behavior, a new polymer design will be necessary based on the non-aggregated polymer approach.¹⁵

3.4 Acknowledgements

I thank Ms. Phoebe Kwan for providing compound 16 (diiodobenzene derivative) and Dr. Zhengguo Zhu for providing compound 10 (diiodoxylene). I also thank Ms. Irina Gorodeskaya for her pyridyl thiophene monomer.
3.5 Experimental procedures

3.5.1 General

UV-vis spectra were recorded by a Hewlett-Packard 8453 diode array spectrometer. Fluorescence spectra were measured by a SPEX Fluorolog-τ2 spectrofluorometer. Quantum yields in water and DMF were determined relative to quinine sulfate solution (0.1N H₂SO₄ aqueous solution, Φ = 0.577). NMR spectra (300MHz) were obtained on Varian Unity or Mercury 300 spectrometer. NMR spectra (500MHz) for compound 9 were obtained on VARIAN Inova-500 NMR spectrometer. High-resolution mass spectra were obtained at the MIT Department of Chemistry Instrumentation Facility (DCIF) on a Bruker Daltronics APEX II 3 Tesla FT-ICR-MS. Diiodohydroquinone (compound 2) was synthesized according to the literature.¹⁶

3.5.2 Monomer synthesis

**Compound 2:** To diiodohydroquinone (compound 1, 15.0g, 41.4mmol) and K₂CO₃ (28.6g, 207mmol) in acetone (100 mL) was added ethyl bromoacetate (17.3g, 104mmol) dropwise. The reaction mixture was refluxed overnight, and the solvent was evaporated off. The crude compound was re-crystallized from ethanol (20.6g, 93%). ¹H NMR (300MHz, CDCl₃): δ 7.16 (s, 2H), 4.14 (s, 2H), 4.30 (q, 4H, J = 7.2Hz), 1.33 (t, 6H, J = 6.9Hz); ¹³C NMR (75MHz, CDCl₃): 166.022, 152.781, 123.714, 86.400, 67.520, 61.794, 14.610; HR-MS (ESI) calcd. for C₁₃H₁₆I₂O₆: 556.8928 (M⁺+Na), found: 556.8928 (M⁺+Na), 1090.7918 (2M+Na).
Compound 3: 2 (4.00g, 7.49mmol) was refluxed with diethanol amine (3.15g, 30.0mmol) in ethanol (50mL) overnight. The solvent was evaporated off, and the white solid was dried under vacuum. After the solid was suspended in dry THF (50mL), imidazole (5.09g, 74.9mmol) and TIPSCI (14.4 g, 74.9mmol) were added, and the reaction mixture was stirred overnight at room temperature. The solvent was removed by a rotary evaporator, and the crude compound was purified by silica gel chromatography and re-crystallization from CH₂Cl₂/MeOH (white crystalline, 8.14g, 85%). ¹H NMR (300MHz, CDCl₃): δ 7.246 (s, 2H), 4.782 (s, 4H), 3.860 (m, 8H), 3.688 (t, 4H, J = 5.1Hz), 3.565 (t, 4H, J = 5.1Hz), 1.046 (m, 84H); ¹³C NMR (75MHz, CDCl₃): 167.468, 153.030, 123.865, 86.407, 69.501, 62.284, 61.753, 51.201, 48.945, 18.316, 18.305, 12.165, 12.133; HR-MS (ESI) calcd. for C₅₄H₁₀₆I₂N₂O₈: 1299.5008 (M+Na), found 1299.5051 (M+Na).

Compound 4: Either of TIPS- or TBDMS-compound 4 afforded the compound 5 by the same procedure. TBAF in THF (1M THF solution, 36mL, 36mmol) was added to TBDMS-protected compound 4 (8g, 7.2mmol). The reaction mixture was stirred overnight, and THF was evaporated off. MeOH/CH₂Cl₂ (20/80, 50mL) was poured into the flask containing the oily residue, and this solution was stirred at room temperature overnight. The resultant white precipitate was collected by a filtration and dried under vacuum (3.26 g, 69%). ¹H NMR (300MHz, DMSO-d₆): δ 7.20 (s, 2H), 4.99 (t, 2H, J = 4.8Hz), 4.92 (s, 2H), 4.71 (t, 2H, J = 5.4Hz), 3.59-3.33 (m, 16H); ¹³C NMR (75MHz, DMSO-d₆): 166.91, 151.82, 122.17,
85.82, 67.17, 58.53, 49.23, 47.7; HR-MS (ESI) calcd. for C_{18}H_{26}N_{2}O_{6}: 652.9851 (M'\textsuperscript{+}H), found: 652.9834 (M'\textsuperscript{+}H), 674.9653 (M'\textsuperscript{+}Na).

**Compound 5:** (i) Compound 4 (19.6g, 15.3mmol) and CuI (58.3 mg, 0.314mmol) were dissolved in a mixture of benzene (120 mL) and Et\textsubscript{3}N (40 mL) in a Schlenk flask. This reaction mixture was degassed by three quick vacuum and back-filled with Ar cycles. PdCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2} (215mg, 0.314mmol) and TMSA (3.08g, 31.4mmol) were added to the flask under Ar. After heating at 50\textdegree C overnight, the solvent was evaporated off and the residue was dissolved in CH\textsubscript{2}Cl\textsubscript{2} and washed with NH\textsubscript{4}Cl aq. solution and dried over MgSO\textsubscript{4}. The crude product obtained by evaporation was re-crystallized from methanol and purified further by silica gel chromatography and re-crystallization from methanol (12.75 g, 68%). \textsuperscript{1}H NMR (300MHz, CD\textsubscript{2}Cl\textsubscript{2}): \delta 6.88 (s, 2H), 4.82 (s, 4H), 3.85 (t, 8H), 3.63 (t, 4H), 3.55 (t, 4H), 1.05 (m, 84H), 0.24 (s, 18H).

(ii) TMS group was removed in the presence of K\textsubscript{2}CO\textsubscript{3} in THF/MeOH. To the compound in procedure (i) (7.52g, 6.17mmol) in a mixture of THF (10mL) and MeOH (10mL) was added K\textsubscript{2}CO\textsubscript{3} (2.13g, 15.4mmol). After stirring overnight, the K\textsubscript{2}CO\textsubscript{3} was filtered off and the filtrate was evaporated. The residue was dissolved in CH\textsubscript{2}Cl\textsubscript{2} and washed with water. The organic layer was dried over MgSO\textsubscript{4} and the solvent was evaporated off after filtration. Re-crystallization from CH\textsubscript{2}Cl\textsubscript{2}/MeOH afforded the desired product (5.67g, 86%). \textsuperscript{1}H NMR (300MHz, CDCl\textsubscript{3}): \delta 7.012 (s, 2H), 4.833 (s, 4H), 3.868 (m, 8H), 3.690 (t, 4H, J = 4.8Hz), 3.585 (t, 4H, J = 5.4Hz), 3.306 (s, 2H), 1.058 (m, 84H); \textsuperscript{13}C NMR

**Compound 6**: TBAF in THF (1M THF solution, 26.4mL, 26.4mmol) was added to compound 5 (5.67g, 5.28mmol) in THF (40 mL). The reaction mixture was stirred overnight, and THF was evaporated off. MeOH/CH₂Cl₂ (20/80, 100mL) was pored to the oily residue and this solution was stirred at room temperature overnight. The resultant white precipitate was collected by a filtration and dried under vacuum (2.16 g, 91%). ¹H NMR (300MHz, DMSO-d₆): δ 6.91 (s, 2H), 5.00 (t, 2H, J = 4.8Hz), 4.94 (s, 2H), 4.71 (t, 2H, J = 5.4Hz), 4.34 (s, 2H), 3.59-3.33 (m, 16H); ¹³C NMR (75MHz, DMSO-d₆): 167.15, 152.89, 116.88, 111.84, 86.06, 79.98, 66.21, 58.61, 49.23, 47.73. HR-MS (ESI) calcd. for C₂₂H₂₈N₂O₆: 449.1918 (M⁺+H), found: 449.1903 (M⁺+H), 471.1725 (M⁺+Na).

**Compound 7**: Compound 2 (8.0g, 15mmol) in MeOH (100mL) was refluxed with NaOH (6.0g, 150mmol) for 2.5 hours. After cooling down, the solvent was evaporated off, and the residue was acidified by 1 N HCl aq. solution. The resultant white precipitate was collected by a centrifugation and washed with water until the pH of the solution became around 4. The collected product was dried under vacuum at 50°C overnight (7g, 98%). ¹H NMR (300MHz, DMSO-d₆): δ 7.232 (s, 2H), 4.737 (s, 4H); ¹³C NMR (75MHz, DMSO-d₆): 169.618, 151.609, 122.149, 86.119, 66.024. HR-MS (ESI) calcd. for C₁₀H₄I₂O₆: 476.8327 (M-H), found: 476.8332.
**Compound 8:** Compound 6 (3.0g, 6.3mmol) in oxalyl chloride (20mL) was refluxed overnight, and the excess oxalyl chloride was distilled off. The residue was dissolved in CH₂Cl₂ (20mL) and cooled down in an ice bath. To this solution, diethyl iminodiacetate (4.8g, 25mmol) in a mixture of CH₂Cl₂ (20 mL) and Et₃N (2.7mL, 19mmol) was added drop wise. The reaction solution was allowed to be room temperature and stirred overnight. This solution was washed with 1N NaOH, 1N HCl and sat. NaCl aq. solutions and then dried over MgSO₄. The solvent was evaporated off after filtration, and the crude product was re-crystallized from ethyl acetate (4.2 g, 81 %). ³¹H NMR (300MHz, CDCl₃): δ 7.232 (s, 2H), 4.727 (s, 4H), 4.308 (s, 4H), 4.193 (m, 12H), 1.265 (t, 12H, J = 7.2Hz); ³¹C NMR (75MHz, CDCl₃): 168.578, 168.418, 167.770, 152.572, 86.044, 69.395, 62.140, 61.641, 49.989, 48.577, 14.434; HR-MS (ESI) calcd. for C₂₆H₃₅I₂N₂O₁₂: 843.0093 (M⁺+Na), found: 843.0113 (M⁺+Na).

**Compound 9:** Small scale; To Compound 8 (50mg, 0.062mmol), Tris (33.1mg, 0.274mmol) and K₂CO₃ (34.4mg, 0.249mmol) in a flask was added DMSO (1 mL) and stirred overnight. Excess solid K₂CO₃ was filtered off, and the filtrate was vacuum-distilled off. The oily residue was rinsed with CH₂Cl₂, and a white precipitate was collected by a centrifuge and dried under vacuum at 60°C (59mg, 85%). Unreacted Tris (53mol% relative to the desired product, 9) was found in ¹H NMR. ¹H NMR (300MHz, DMSO-d₆): δ 7.731 (bs, 2H), 7.423 (bs, 2H), 7.226 (bs, 2H), 4.845 (bs, 4H),
4.617 (bs, 12H), 4.064 (bs, 8H), 3.945 (bs, 4H), 3.587 (bs, 12H), 3.545 (bs, 12H) (D$_2$O): 7.213 (bs, 2H), 4.884 (bs, 4H), 4.284 (bs, 4H), 4.101 (bs, 4H), 3.736 (bs, 24H). HR-MS (ESI) calcd. for C$_{34}$H$_{54}$I$_2$N$_6$O$_{20}$: 1143.1374 (M$^+$+Na), found: 1143.1377 (M$^+$+Na)

Large scale: Compound 8 (1.00g, 1.24mmol), Tris (0.602g, 4.97mmol) and K$_2$CO$_3$ (0.687g, 4.97mmol) was added DMSO (10mL) and stirred overnight. Excess solid K$_2$CO$_3$ was filtered off, and the filtrate was vacuum-distilled off. After rinsing the oily residue with CH$_2$Cl$_2$, the precipitate was collected by a centrifuge and dried under vacuum. This crude product was dissolved in a small amount of water and precipitated in EtOH (0.90g, 65%). Unreacted Tris (17mol% relative to the desired product, 9) was found in $^1$H NMR. $^1$H NMR (500MHz, DMSO-$d_6$): $\delta$ 7.748 (bs, 2H), 7.443 (bs, 2H), 7.24 (bm, 2H), 4.855 (bs, 4H), 4.628 (bs, 12H), 4.075 (bs, 4H), 3.953 (bs 4H), 3.603 (bs, 12H), 5.593 (bs, 12H). $^{13}$C NMR (125MHz, DMSO-$d_6$): 169.276, 168.699, 168.406, 151.945, 122.333, 85.881, 66.674, 62.794, 62.558, 60.279, 59.752, 51.050. HR-MS (ESI) calcd. for C$_{34}$H$_{54}$I$_2$N$_6$O$_{20}$: 1121.1555 (M$^+$+H), found: 1121.1531 (M$^+$+H).

**Compound 11:** Compound 10 (1.0g, 2.8mmol), NBS (0.99g, 5.6mmol) and AIBN (4.6mg, 0.028mmol) was dispersed in benzene (5 mL) and refluxed for 2.5 hours. After cooling down to room temperature, the resultant precipitate was collected by a filtration and washed with benzene and MeOH and dried under vacuum (0.33g, 23%). This crude product was used for the following step. $^1$H NMR (300MHz, CDCl$_3$): $\delta$ 7.91 (s, 2H), 4.49 (s, 4H).
Compound 12: Compound 11 (200mg, 0.388mmol), K$_2$CO$_3$ (214mg, 1.55mmol) and triester (360mg, 1.55 mmol) were placed in 25 mL Schlenk flask. DMF (1mL) was added to the flask, and the reaction mixture was stirred at 50°C. After completion of the reaction confirmed by $^1$H NMR, the reaction mixture was diluted with CH$_2$Cl$_2$ and washed with water. The aqueous layer was extracted by CH$_2$Cl$_2$. The combined organic layer was washed with 1N NaOH and sat. NaCl aq. solutions and dried over MgSO$_4$. The crude product was obtained by evaporation of the solvent after filtration and purified by silica gel chromatography (ethyl acetate(40)/hexane(60)) (300mg, 94%). $^1$H NMR (300MHz, CDCl$_3$): δ 7.77 (s, 2H), 4.25 (m, 12H), 3.79 (s, 4H), 4.25 (t, 18H).

General procedure for amidation: Compound 13, Compound 15, Compound 18; The starting compound (1eq.) K$_2$CO$_3$ (6eq.) and Tris (6eq.) were placed in oven-dried 25mL Schlenk tube under Ar. K$_2$CO$_3$-dried DMSO was added to the tube under Ar, and the reaction mixture was stirred at room temperature or 50°C overnight. After cooling down, excess solid K$_2$CO$_3$ was filtered off, and DMSO was distilled off. By rinsing the residue with CH$_2$Cl$_2$, a white precipitate was obtained and dried under vacuum.

Compound 14: Compound 7 (200mg, 0.418mmol) was refluxed in thionylchloride (3mL, 41mmol) overnight, and then excess thionylchloride was distilled off. The residue in DMF (1 mL) was added to the diethyl aminomalonate HCl in DMF (3 mL) and Et$_3$N (0.41mL). The reaction mixture was stirred at room temperature overnight and taken
up by CH$_2$Cl$_2$ and washed with water, sat. NH$_4$Cl and sat. NaCl aq. solutions and dried over MgSO$_4$. The crude product was purified by a silica gel chromatography (CH$_2$Cl$_2$ (90)/EtOAc (10)) (124 mg, 37.3%). $^1$H NMR (300MHz, CDCl$_3$): δ 7.91 (d, 2H, J = 6.3Hz), 7.19 (s, 2H), 5.26 (d, 2H, J = 6.9Hz), 4.54 (s, 4H), 4.31 (m, 8H), 1.32 (t, 12H, J = 7.2Hz).

**Compound 17:** Compound 16 (100mg, 0.127mmol), K$_2$CO$_3$ (71mg, 0.51mmol) and triester (119mg, 0.512mmol) were placed in oven-dried 25 mL Schlenk tube under Ar. To this tube, DMF was added, and the reaction mixture was stirred at 50°C overnight. The reaction solution was filtered, and the filtrate was washed with water. The aqueous layer was extracted with EtOAc.

The combined organic layer was washed with 1N NaOH and sat. NaCl aq. solutions and dried over MgSO$_4$. The crude product was purified by a silica gel chromatography (EtOAc(30)/hexane(70)) (109mg, 86%). $^1$H NMR (300MHz, CDCl$_3$): δ 7.22 (s, 2H), 4.26 (q, 12H, J = 7.2Hz), 4.05 (t, 4H, J = 5.1Hz), 3.82 (m, 8H), 2.50 (t, 4H, J = 6.9Hz), 1.28 (t, 18H, J = 7.2Hz).

**Compound 19:** Ethyl 3-thiopeneacetate (3.0g, 18mmol) and NBS (6.9g, 39mmol) were dissolved in DMF (20mL). This reaction mixture was stirred at room temperature overnight and diluted with CH$_2$Cl$_2$ and washed with 1N NaOH.
and \( \text{NH}_4\text{Cl} \) aq. solutions and dried over MgSO\(_4\). The crude product was purified by a silica gel chromatography (EtOAc(20)/hexane(80)) (5.5g, 95%). \(^1\)H NMR (300MHz, CDCl\(_3\)): \( \delta \) 6.94 (s, 2H), 4.19 (q, 2H, J = 7.2Hz), 3.56 (s, 2H), 1.28 (t, 3H, J = 7.2Hz).

**Compound 20:** DMSO (5mL) was added to the flask containing Compound 19 (1.00g, 3.04mmol), Tris (400mg, 3.36mmol) and \( \text{K}_2\text{CO}_3 \) (464mg, 3.36mmol). The reaction mixture was stirred overnight, and the excess \( \text{K}_2\text{CO}_3 \) was filtered off. The filtrate was distilled, and the oily residue was rinsed with CH\(_2\)Cl\(_2\). The resultant precipitate was dissolved in DMF and precipitated in CH\(_2\)Cl\(_2\) (863mg, 70%). \(^1\)H NMR (300MHz, DMSO-\( d_6 \)): \( \delta \) 7.42 (s, 1H), 7.14 (s, 1H), 4.65 (s, 3H), 3.45 (s, 2H), 3.33 (s, 6H).

**Compound 21:** DCC (0.87g, 4.2mmol) was added to DMF solution (5mL) containing 3-thiopheneacetic acid (500mg, 3.52mmol), diethyl iminodiacetate (1.0g, 5.3mmol) and DMAP (130mg, 1.06mmol). The solution was stirred at room temperature overnight. The precipitate was filtered off, and the filtrate was washed with 1N NaOH, 1N HCl, 10% NaHCO\(_3\) and sat. NaCl aq. solutions and dried over MgSO\(_4\). The crude product was purified by a silica gel chromatography (EtOAc(10)/CH\(_2\)Cl\(_2\) (80)) (oil. 0.79g, 72%). \(^1\)H NMR (300MHz, CDCl\(_3\)): d 7.31 (m, 1H), 7.18 (m, 1H), 7.03 (m, 2H), 4.24 (m, 8H), 3.76 (s, 2H), 1.28 (m, 6H).

**Compound 22:** Compound 21 (0.50g, 1.6mmol) and NBS (0.63g, 3.5mmol) were dissolved in DMF (5mL). This reaction mixture was stirred at room temperature
overnight. The reaction solution was diluted with CH₂Cl₂ and washed with water. The aqueous layer was extracted with CH₂Cl₂ (50mL x 2). The combined organic layer was washed with 1N NaOH and sat. NaCl aq. solutions and dried over MgSO₄. The crude product was purified by a silica gel chromatography (EtOAc(5)/CH₂Cl₂ (95)) (0.7g, 93%). ¹H NMR (300MHz, CDCl₃): δ 6.98 (s, 1H), 7.18 (m, 1H), 4.22 (m, 8H), 3.63 (s, 2H), 1.30 (m, 6H).

**Compound 23:** DMSO (2mL) was added to the flask containing Compound 22 (0.60g, 1.3mmol), Tris (0.31g, 2.6mmol) and K₂CO₃ (0.35g, 2.6mmol). The reaction mixture was stirred overnight, and the excess K₂CO₃ was filtered off. The filtrate was distilled, and the oily residue was rinsed with CH₂Cl₂. ¹H NMR (300MHz, D₂O): δ 6.95 (s, 1H), 4.40 (s, 1H), 4.12 (s, 1H), 4.10 (s, 2H), 3.84 (s, 1H), 3.81 (s, 12H).

### 3.5.3 Polymer synthesis

**Synthesis of Polymer A:** Monomer 6 (12.4mg, 0.0276mmol) and 9 (30.0mg, 0.0268mmol) were placed in 25mL Schlenk flask. Pd(PPh₃)₄ (5%) and CuI (5%) were added to the flask in a glove box. Morpholine was passed through an alumina column and degassed by three quick cycles of vacuum and back-filling with Ar and cannulated into the flask containing the monomers (~0.5mL). The reaction mixture was also degassed by the same procedure and stirred at 60°C overnight and poured into EtOAc. The precipitate was collected by a centrifuge and washed with EtOAc and
diethyl ether and dried under vacuum at 60°C. This polymer was used for GPC characterizations. A part of the compound was dissolved in water and dialyzed against water. The polymer solution was lyophilized.
Compound 2:
$^1$H-NMR (300MHz, CDCl$_3$)

$^{13}$C-NMR (75MHz, CDCl$_3$)
Compound 3:
$^1$H-NMR (300MHz, CDCl$_3$)

$^{13}$C-NMR (75MHz, CDCl$_3$)
Compound 4:
$^1$H-NMR (300MHz, DMSO-$d_6$)

$^{13}$C-NMR (75MHz, DMSO-$d_6$)
Compound 5:
$^1$H-NMR (300MHz, CDCl$_3$)

$^{13}$C-NMR (75MHz, CDCl$_3$)
**Compound 6:**

\(^1\)H-NMR (300MHz, DMSO-\(d_6\))

\(^13\)C-NMR (75MHz, DMSO-\(d_6\))
Compound 7:
$^1$H-NMR (300MHz, DMSO-$d_6$)

$^{13}$C-NMR (75MHz, DMSO-$d_6$)
Compound 8:

$^1$H-NMR (300MHz, CDCl$_3$)

$^{13}$C-NMR (75MHz, CDCl$_3$)
Compound 9 (Small scale):
$^1$H-NMR (300MHz, DMSO-$d_6$)

$^1$H-NMR (300MHz, D$_2$O)


**Compound 9 (Large scale):**

$^1$H-NMR (500MHz, DMSO-$d_6$)

$^{13}$C-NMR (125MHz, DMSO-$d_6$)
Compound 11: $^1$H-NMR (300MHz, CDCl$_3$)

Compound 12: $^1$H-NMR (300MHz, CDCl$_3$)
Compound 14: $^1$H-NMR (300MHz, CDCl$_3$)

Compound 17: $^1$H-NMR (300MHz, CDCl$_3$)
Compound 19: $^1$H-NMR (300MHz, CDCl$_3$)

Compound 20: $^1$H-NMR (300MHz, DMSO-$d_6$)
Compound 21: \(^1\text{H-}\text{NMR (300MHz, CDCl}_3\text{)}\)

Compound 22: \(^1\text{H-}\text{NMR (300MHz, CDCl}_3\text{)}\)
Compound 23: $^1$H-NMR (300MHz, D$_2$O)
3.6 References

3 S. Shi and F. Wudl, Macromolecules, 1990, 23, 2119
Chapter 4: Atom transfer radical polymerization of N-isopropylacrylamide

Abstract: Poly(N-isopropylacrylamide) (polyNIPA) has been synthesized by atom transfer radical polymerization (ATRP) at room temperature in benzene or DMF using α-chloroester or α-chloroamide initiators and a CuCl/tris[2-(dimethylamino)ethyl]amine (Me₃TREN) catalyst. The crude yield was more than 60%, and polydispersity is less than 1.5 for most of the cases. Poly(phenylene-ethynylene)-graft-polyNIPA has been also synthesized via this ATRP method.
4.1 Introduction

In the studies of synthetic route to polyNIPA-modified conjugated polymers, we have developed atom transfer radical polymerization (ATRP) of NIPA. Based on the ATRP method, we have designed synthetic approaches toward PPE-polyNIPA copolymers. The conjugated polymers are modified with ATRP initiator and polyNIPA can be grown by radical polymerization. Another route is that polyNIPA is polymerized using an initiator based upon iodophenyl derivatives, and thus functionalized polyNIPA was used to endcap a PPE polymerization. The details of the block copolymerizations are described in Chapter 5. In this chapter, ATRP conditions for NIPA polymerization and characterization of the obtained polyNIPA including end group functionalities are discussed.

\[
\begin{align*}
\text{NIPA} & \quad \text{PolyNIPA} \\
\end{align*}
\]

4.1.1 Atom transfer radical polymerization (ATRP) and its mechanism

Atom transfer radical polymerization (ATRP) enables the creation of well-defined polymers with mono-disperse molecular weights and functionalities at the end-group or on the polymer side chains that lead to the design of new polymeric architectures such as block-, breached- and graft polymers.\(^{12}\) The classical methods to obtain such polymers make use of ionic polymerizations, which limit the nature of the monomers due to the reactivity of the ionic intermediate and cross-reactions between functional groups during polymerization and further require extreme exclusion of oxygen, water and typical solvent impurities. In contrast, radical polymerizations are tolerant of many functionalized
monomers and are compatible with many solvents and thereby provide facile access to functional polymeric materials. In addition, radical polymerizations are also tolerant to many impurities, which has made them suitable for industrial large-scale production. However, the reactivity of radical intermediate is difficult to control because of irreversible termination reactions.

Scheme 4-1

**Initiation:**
\[
I^\cdot + Cu(I)/L \xrightarrow{=} I^\cdot + X-Cu(II)/L \quad (1)
\]

**Propagation:**
\[
I-P_m^\cdot X + Cu(I)/L \xrightarrow{+M} I-P_m^\cdot + X-Cu(II)/L \quad (2)
\]

**Termination:**
\[
I-P_m^\cdot + I-P_n^\cdot \xrightarrow{+M} I-P_{m+n}^{-1} \quad (3)
\]

The ATRP method is employed to avoid unfavorable termination reactions in radical polymerization by controlling the equilibrium between radical and dormant (non-radical) forms in the presence of a metal catalyst. The mechanism of ATRP utilizing a copper ion as a metal catalyst is shown in Scheme 4-1. First, an initiator (I-X) produces a radical (I•) when the Cu(I)/L abstracts a halogen atom (X) from I-X to produce a X-Cu(II)/L species. The generated initiator radical (I•) reacts with monomers, and the propagation of polymer chain begins. The radical at the chain end (I-P_m•) is deactivated by the conversion of X-Cu(II)/L to Cu(I)/L and the halogenation of the polymer (I-P_m-X). The continued propagation of a polymer chain is made possible by the repetition of this reversible activation/deactivation process of radical species, and ideally the reaction
persists until all of the monomers are consumed. The fact that the equilibrium between radical and dormant forms (ATRP equilibrium in (2)) is shifted to the dormant form, eliminates termination processes involving radical-radical coupling because of a low radical concentration during polymerization. Multi-dentate amines have been used as ligands for the Cu ion to stabilize the Cu(II) species and to provide solubility in organic solvents. The redox potential of the Cu ion is altered by complexation with the ligand so that the catalytic activity is tunable depending on the monomers and conditions.

The initiation rate is also important to obtain well-defined polymers. The initiation should be fast allowing the polymer chains to begin growing at the same time; otherwise, the chain lengths will be of very different lengths because the polymers, ideally, grow with the same propagation rate. However, if the initiation rate is too large, the large number of radical species is produced in the initial stage, resulting in radical-radical termination. Practically, the chemical structure of an initiator is chosen to be the same as the monomer to avoid mismatching of radical reactivity with monomers. The ATRP equilibrium and the initiation conditions are primary factors to control the polymerization and should be considered in the design of the monomers, catalysts and polymers.

4.1.2 Previous studies on ATRP of acrylamide derivatives

ATRP has been successful in the polymerization of traditional monomers such as styrene and acrylate derivatives. However, the polymerization of acrylamide derivatives is still difficult to control, and experimental investigation continues. The number of reports for poly(acrylamide derivative)s is limited relative to those for polystyrene or
polyacrylate. The following sections review the ATRP of acrylamide and its derivatives including N-isopropylacrylamide (NIPAA).

4.1.3 Acrylamide

Unmodified acrylamide has been polymerized using an initiator confined on the surface or in solution.\textsuperscript{3,4,5,6} Huang et al. made a polyacrylamide (polyAAm) film from benzyl chloride attached on silica gel surface in the presence of CuCl and bpy in DMF at 130°C.\textsuperscript{3,4,5} The polyAAm was cleaved from the surface and characterized by MALDI-TOF-MS, which showed a narrow molecular weight distribution; however, no detailed characterization in respect with controlled polymerization was provided.\textsuperscript{5}

\[\text{H}_2\text{N}^\equiv\text{O}\]

\[\text{N}^\equiv\text{N}\]

AAm bpy

4.1.4 N, N-dimethylacrylamide

ATRP of N, N-dimethylacrylamide (DMAAm) is difficult to control because of two potential side reactions: inactivation of a Cu catalyst by complexation with the amide group of DMAAm and loss of the terminal halogen group by the nucleophilic cyclization reactions with the amide groups.\textsuperscript{7,8,9} To prevent these side reactions, Teodorescu et al. proposed to use alkyl chlorides rather than bromides, Cu/tris[2-(dimethylamino)ethyl]amine (Me\textsubscript{6}TREN) (which is powerful enough for polymerization at room temperature) and low polar solvents (which reduce the cyclization reactions).\textsuperscript{9} Indeed, in accordance to their hypothesis, they obtained polyDMAAm with low polydispersity (1.11) although the yield was less than 60%.
4.1.5 \textit{N}-isopropylacrylmaide and other acrylamide derivatives

Free radical polymerization is the most common way to make polyNIPA.\textsuperscript{10} Zhu \textit{et al.} reported synthesis of asymmetric block copolymers of polyNIPA and dendritic polyether by ATRP of NIPA.\textsuperscript{11,12} They polymerized NIPA using dendritic poly(benzyl ether) bromides as an initiator and CuBr/1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane (Me\textsubscript{4}Cyclam) as a catalyst in THF at 90°C. They observed the formation of nanotubules from spherical aggregates by heating. Teodorescu \textit{et al.} demonstrated ATRP of \textit{N}-(hydroxypropyl)-methacrylamide (HPMA) and \textit{N}-tert-butylacrylamide (t-BAA) in the same procedure for DMAA.\textsuperscript{9} Although their polydispersities are low (< 1.3), the conversions were limited (18.5% for HPMA and 38% for t-BAA). ATRP of acrylamide has not been satisfactorily achieved; however, other methods have produced controlled polymerization of NIPA and other AAm derivatives. PolyNIPA with narrow molecular weight distribution has been synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization using a conventional azo-initiator (AIBN) or peroxide in the presence of dithioester derivatives such as benzyl dithionenzoate (BDTB).\textsuperscript{13} Arotçaréna \textit{et al.} reported synthesis of a block copolymer of NIPA and a zwitter ionic monomer using the RAFT method.\textsuperscript{14} This block copolymer forms a micelle in water and has double temperature-responsive properties.
4.1.6 ATRP design of NIPA

Following Teodorescu and Matyjaszewski’s study on ATRP of acrylamide derivatives, we applied the same conditions for the polymerization of NIPA with the following: (1) a chloride initiator, (2) a CuCl/Me₆TREN catalyst and (3) benzene and DMF as solvents. These conditions are expected to reduce replacement of terminal chloride with amide nitrogen (Sₐ2 reaction). In addition to ATRP of NIPA, this project aims to synthesize block copolymers of polyNIPA with conjugated polymers, and thus an initiator with end-cap groups containing an iodophenyl group was prepared and characterized.
4.2 Results and discussion

4.2.1 ATRP of NIPA

ATRP of NIPA was carried out using CuCl/Me₆TREN as the catalyst with methyl 2-chloropropionate (MCP), 4-iodobenzyl bromide (IBBr), compound 1 and compound 2 as initiators. The attempted polymerization conditions are summarized in Table 4-1. The crude yield was more than 60%, and polydispersity is less than 1.5 for most of the cases.

![Chemical structures](image)

Table 4-1. ATRP of NIPA

<table>
<thead>
<tr>
<th>No.</th>
<th>Initiator</th>
<th>Solvent</th>
<th>Mn* (Mn (theory))</th>
<th>Mw*</th>
<th>Mw/Mn</th>
<th>Yield(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MCP</td>
<td>Benzene</td>
<td>14,404 (11,439)</td>
<td>17,358</td>
<td>1.21</td>
<td>70%</td>
</tr>
<tr>
<td>2</td>
<td>MCP</td>
<td>DMF</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>66%</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>DMF</td>
<td>Run#1: 18,130 (11,785)</td>
<td>19,804</td>
<td>1.09</td>
<td>64%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Run#2: 16,559 (11,785)</td>
<td>28,812</td>
<td>1.74*</td>
<td>(40%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Run#3: 16,473 (11,785)</td>
<td>20,862</td>
<td>1.27</td>
<td>72%*(^{60%})</td>
</tr>
<tr>
<td>4</td>
<td>IBBR</td>
<td>Benzene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>No polym.</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>DMF</td>
<td>10,686 (11,754)</td>
<td>14,314</td>
<td>1.34</td>
<td>38%</td>
</tr>
</tbody>
</table>

Conditions: NIPA: initiator: CuCl: Me₆TREN = 100:1:1:1, room temperature, overnight, a) GPC (eluent: DMF, PMAA standard), b) before alumina purification, c) total yield after alumina purification. * GPC elution curve showed a small shoulder peak in the high molecular region.
4.2.2 Polymerization and purification

The ATRP of NIPA was employed in benzene or DMF at room temperature overnight. The color of polymerization mixture changed from blue to green after overnight, which suggests that Cu (II) was formed in the completion of the reaction after overnight, and therefore, no active radical species remained. The polymer was precipitated in ethyl ether and a residual blue color in the crude polymer indicates that the polymer still contains Cu ions, perhaps due to complexation with amide groups in polyNIPA. The crude polymer was dissolved in acetone and stirred with an alumina powder or passed through an alumina column, and the alumina powder became blue-green. After removing the alumina powder by filtration, the polymer was again precipitated in ethyl ether. The yield of this alumina purification step is about 80%, and the resultant polymer was a white powder. Figure 4-1 shows the GPC (gel permeation chromatography) elution curves of polyNIPA (No. 3, Run#1, Table 4-1) before and after alumina purification. The crude polyNIPA containing a Cu ion showed a major large peak and a shoulder peak in shorter retention time or high molecular weight region (Figure 4-1 (a)). A Cu-complex of amide groups in polyNIPA likely non-covalently cross-linked the polymer chains to produce high molecular weight aggregates. On the other hand, the purified polymer showed a single peak (Figure 4-2 (b)). One may think that high molecular weight polymers were selectively removed by alumina, resulting in low poly-dispersity; however, the major peaks in the both GPC elution curves have the same retention time. If the higher molecular weight polymers were adsorbed, the peak should shift to the longer retention time or lower molecular weight region. Hence polyNIPA undergoes non-selective absorption by alumina.
4.2.3 End-group analysis

In the ATRP mechanism, the end-group functionalities, chloride in this case, are expected to be found after polymerization, and they will be useful to elucidate the reaction mechanism and design further synthetic extensions. Hence, the characterization of the oligomer (the degree of polymerization is 10-20) was carried out by $^1$H NMR and MALDI-MS. The oligomer was prepared by higher ratio of initiator 1 and NIPA in the same procedure before.

The NMR spectra of initiator 1 and oligomer are shown in Figure 4-2. The characteristic quartet peak of the proton Ha is observed at 4.5 ppm for the initiator in Figure 4-2, A. In the case of the oligomer (Figure 4-2 B), the spectrum has no peak from the proton Hb that is expected to be around 4.5 ppm although the initiator was intact during radical polymerization. In Figure 4-3, MALDI-MS spectrum has no signals matching the molecular weights of the expected chloride-terminated oligomers. If the terminal hydrogen was removed by hydrolysis, the molecular weights should be those of hydroxyl group-terminated oligomers. However, the terminal halogen could not be
observed depending on an ionization condition in MS procedure.\textsuperscript{16} The possible scenarios are:

(1) The oligomer loses the chlorine atom in the terminal group by an irreversible radical deactivation/termination process involving radical transfer to a solvent or radical-radical coupling;

(2) The chlorine atom was removed by precipitation because the terminal group was still active;

(3) Alumina removed the chlorine atom from the oligomer.

In these cases, the terminal group could be H or OH, but the MS analysis does not support these hypotheses. A detailed analysis is necessary in order to get insight into the reaction mechanism. Radical quenching by silyl enol ether compounds during polymerization could be helpful for the analysis.\textsuperscript{16,17} Ando \textit{et. al} reported that silyl enol ethers such as \textit{p}-methoxy-\textit{α}-(trimethylsiloxy)styrene react with the radical at the chain end of poly(methyl methacrylate) (polyMMA) to terminate the polymerization (Scheme 4-2).\textsuperscript{17} The incorporation of aromatic ketone groups as the polymer ends facilitates characterization by \textsuperscript{1}H-NMR and MALDI-MS.\textsuperscript{16}

\textbf{Scheme 4-2}

![Scheme 4-2](image)

Therefore, if the chain end of polyNIPA is still an active radical during the polymerization, then, a phenyl ketone will be quantitatively incorporated into the chain end. This ketone group can be confirmed by MS analysis. In addition, this method can
be utilized to selectively attach further functional groups on the polymer end using this method.

Figure 4-2. NMR spectra (DMSO-$d_6$) of the initiator 1 (A) and oligoNIPA (B)
4.2.4 Synthesis of PPE-graft-polyNIPA

One of the advantages of ATRP is that it is easy to attach initiators on polymers or to the surface of materials. As a model study, graft-polymerization of NIPA from a conjugated polymer, poly(phenylene-ethynylene) (PPE), was attempted. Polymer A was modified with 2-chloropropionic acid by DCC coupling, and a macroinitiator Polymer B was obtained. NIPA was polymerized in the same ATRP condition as before in the presence of Polymer B. Polymer A and C were characterized by GPC (eluent: DMF; PMAA standard) (Table 4-2). Polymer C showed two distinguished peaks. The minor peak in lower molecular weight region may be free polyNIPA rather than unmodified conjugated polymers because even small amount of initiator attached on Polymer A should make the molecular weight of the graft polymers large and the molecular distribution contentious and broad. Based upon the molecular weights of Polymer A and
C and the molecular weight of grafted polyNIPA of 344,848, the is estimated to be 139 per a repeating unit of the PPE or 23 per an initiator (the theoretical degree of polymerization is 100 per an initiator). Polymer C is highly water-soluble and very fluorescent, whereas Polymer A is slightly soluble in water, and the fluorescence is weak. The solution of Polymer C showed phase separation by heating, and the resultant precipitate was fluorescent. These results support that polyNIPA was successfully grafted on the conjugated polymer.

**Scheme 4-3**

![Scheme 4-3](image)

**Table 4-2.** Molecular weight of Polymer A and C (eluent: DMF), PMAA standard

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Mn</th>
<th>Mw</th>
<th>n&lt;sup&gt;e&lt;/sup&gt;</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>21,625</td>
<td>45,133</td>
<td>22</td>
<td>2.09</td>
</tr>
<tr>
<td>C&lt;sup&gt;a&lt;/sup&gt;</td>
<td>366,473&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>445,345&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>17,326</td>
<td>29,243</td>
<td>-</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Conditions: NIPA: initiator: CuCl: Me<sub>3</sub>TREN = 100:1:1:1, DMF, room temperature. Mn and Mw were obtained from PMMA standard. a) two peaks are observed. b) main peak c) degree of polymerization, d) Mn (theory) = 1.5 × 10<sup>5</sup>
4.3 Conclusion

PolyNIPA with a low poly-dispersity has been synthesized by ATRP using CuCl/Me₄TRN catalyst at room temperature in benzene or DMF. This method was successful to functionalize the terminal end of polyNIPA with iodophenyl groups or fluorescein. The polymerization at room temperature allows the incorporation of functionalized compounds due to the lack of side reactions. The ATRP method was extended to synthesis of graft polymers with a rigid rod-like conjugated polymer core and polyNIPA graft. This copolymer is water-soluble and thermally responsive, without loosing the fluorescence of the conjugated polymer. This approach can be used to make polyNIPA grafted from a surface of materials. Moreover, ATRP synthesis of NIPA provides well-defined materials that can be used for study of its solution properties and to gain insight into the mechanism of thermal phase separation.
4.4 Experimental procedures

4.4.1 General

NMR spectra were obtained on Varian Unity or Mercury 300 spectrometer. High-resolution mass spectra and MALDI-MS spectrum were obtained at the MIT Department of Chemistry Instrumentation Facility (DCIF) on a Bruker Daltronics APEX II 3 Tesla FT-ICR-MS. Methyl 2-chloropropionate and 4-iodobenzyl bromide were purchased from Alfa-Aesar and used without further purification. CuCl was stirred in acetic acid overnight. The remaining solid was filtrated, washed with EtOH and dried under vacuum. The purified CuCl (gray powder) was stored under Ar in a Schlenk tube. NIPA was purified by re-crystallization from toluene/hexane.

4.4.2 Initiators

**Compound 1:**

![Chemical Structure]

**Compound 3** was prepared from 4-iodobenzoic acid and 2, 2'-(ethylenedioxy) bis(ethylamine). 2, 2'-(ethylenedioxy) bis(ethylamine) (22.2g, 150mmol) in CH$_2$Cl$_2$ (150mL) was added drop wise to 4-Iodobenzoic chloride (5.00g, 18.8mmol) in CH$_2$Cl$_2$ (125mL) and Et$_3$N (5mL). The reaction mixture was stirred overnight and evaporated off. Water was added to the residue, and the resultant precipitate was filtered off. The filtrate was extracted with EtOAc (400mL). The organic layer was dried over MgSO$_4$ and evaporated off after a filtration. The oily residue was solidified under vacuum. $^1$H NMR (300MHz, DMSO-$d_6$): δ 8.621 (t, 1H, $J = 6.3$Hz), 7.82 (d, 2H, $J = 8.4$Hz), 7.63 (d, 2H, $J = 8.7$Hz), 4.25-3.45 (m, 6H), 3.59-3.33 (m, 4H), 2.64 (t, 2H, $J = 5.7$Hz), 2.34 (bs,
$^{13}$C NMR (75MHz, DMSO-$d_6$): 165.34, 136.94, 133.61, 129.01, 98.68, 72.90, 69.57, 69.47, 68.77, 41.31; HR-MS (ESI) calcd. for C$_{13}$H$_{19}$IN$_2$O$_3$: 379.0513 (M$^+$+H), found: 379.0506 (M$^+$+H), 401.0356 (M$^+$+Na).

2-Chloropropionic chloride (0.71g, 5.6mmol) was added to compound 3 (2.0g, 5.3mmol) in a mixture of CH$_2$Cl$_2$ (20mL) and Et$_3$N (0.74mL, 5.3mmol) on an ice bath. The reaction mixture was allowed to be room temperature and stirred overnight. The solvent was evaporated off and the crude product was purified by a silica gel chromatography (CH$_2$Cl$_2$ (95)/MeOH (5)) (2.3g, 91%). $^1$H NMR (300MHz, CDCl$_3$): $\delta$ 7.88 (d, 2H, $J = 6.6$Hz), 7.53 (d, 2H, $J = 6.9$Hz), 6.98 (bs, 1H), 6.76 (bs, 1H), 4.44 (q, 1H, $J = 7.2$Hz), 4.25-3.43 (m, 12H), 1.75 (d, 3H, $J = 6.9$Hz); $^{13}$C NMR (75MHz, DMSO-$d_6$): 169.76, 166.88, 137.87, 133.99, 128.81, 98.71, 70.59, 70.54, 70.01, 69.71, 56.21, 40.18, 39.96, 23.07; HR-MS (ESI) calcd. for C$_{16}$H$_{22}$ClN$_2$O$_4$I: 491.0205 (M$^+$+H), found: 469.0397 (M$^+$+H), 491.0197 (M$^+$+Na).

**Compound 2:**

![Diagram of Compound 2]

2-Chloropropionic chloride (0.37g, 2.9mmol) was added dropwise to fluorescein amine (500mg, 1.44mmol) in a mixture of acetone (10mL) on an ice bath. Yellow white precipitate formed immediately after addition of chloride. After 3 hours, the precipitate was collected by a filtration and stirred in acetone again. The solid was collected by a filtration, washed with CH$_2$Cl$_2$ and acetone and dried under vacuum (447mg, 71%). $^1$H
NMR (300MHz, DMSO-d$_6$): $\delta$ 10.92 (s, 1H), 8.34 (s, 1H), 7.90 (m, 1H), 7.27 (d, 1H, $J = 8.7$Hz), 6.69 (d, 2H, $J = 1.5$Hz), 6.63 (m, 4H), 4.77 (q, 1H, $J = 6.9$Hz), 1.67 (d, 3H, $J = 6.6$Hz).

**Tris[2-(dimethylamino)ethyl]amine (Me$_3$TREN)$_{18}$**

A solvent mixture was prepared by slowly pouring formic acid (24mL, 62mmol) and formaldehyde (37%, 24mL, 29mmol) to water (6mL). Tris (2-aminoethyl)amine (6g, 41mmol) was added to the solvent and heated at 120°C with a condenser overnight. The mixture was condensed by evaporation, and 10% NaOH aq. solution (100mL) and 20% KOH (100mL) was added to the oily residue. This aqueous solution was extracted by ethyl ether (50mL x 4). Evaporation of the solvent gave the crude product (oil, 5.85g) that was purified by a vacuum distillation using a Kugelrohr distillatory apparatus (4.41g, 46.7%). $^1$H NMR (300MHz, CDCl$_3$): $\delta$ 2.60 (m, 6H), 2.40 (m, 6H), 2.22 (s, 18H).

### 4.4.3 Polymerization

**Typical procedure (No.3 in Table 1):**

![Polymerization Reaction](image-url)
NIPA (500mg, 4.42mmol) and CuCl (4.4mg, 0.044mmol) were placed in 25mL Schlenk tube and degassed by three vacuum-Ar filling cycles. Me₆TREN (10.2mg, 0.042mmol) in DMF (1mL) was poured to the tube. After 5 minutes, initiator 1 (20.7mg, 0.0442mmol) in DMF (0.5mL) was added to the mixture. The reaction solution was stirred overnight and dropped into ethyl ether (70mL). The obtained gummy product was dissolved in DMF and precipitated in ethyl ether again. Green precipitate was collected by a centrifugation and dried under vacuum. The product was stirred with alumina in acetone for 4 hours. The alumina was filtered off, and the filtrate was concentrated under reduced pressure and dropped into ether. The resultant white precipitate was collected by a centrifugation and dried under vacuum.

4.4.4 Synthesis of conjugated polymer-graft-polyNIPA (Polymer C)

Polymer A (5.0mg) was dissolved in small amount of DMF, and the solvent was evaporated at 50°C under vacuum for 2 hours to remove water from the polymer. Dry DMF (0.3mL), DCC (20.6mg, 0.100mmol), DMAP (1.2mg, 0.010mmol) and 2-chloropropionic acid (10.9mg, 0.100mmol) were added to the polymer under Ar. The solution was stirred overnight, diluted by CH₂Cl₂ and filtered. The filtrate was evaporated off, and dried under vacuum at 40°C (Polymer B). Me₆TREN (4.2mg, 0.018mmol) in DMF (0.8mL) was added to NIPA (204mg, 1.80mmol) and CuCl (1.8mg, 0.018mmol) in a Schlenk tube under Ar. After 5 minutes, Polymer B (3.4mg) in DMF (0.2mL) was added to the reaction mixture. The solution was stirred overnight, and the solvent was distilled off. The residue was dissolved in small amount of acetone and
precipitated in ethyl ether. The yellow precipitate was collected by a centrifugation and
dried under vacuum (Polymer C, 106.4mg).
Compound 1:

$^1$H-NMR (300MHz, CDCl$_3$)

$^{13}$C-NMR (75MHz, CDCl$_3$)
Compound 3:

$^1$H-NMR (300MHz, DMSO-$d_6$)

$^{13}$C-NMR (75MHz, DMSO-$d_6$)
4.5 References

Chapter 5: Synthesis of thermally responsive poly(phenylene-ethynylene) via atom transfer radical polymerization of N-isopropylacrylamide

Abstract: Synthesis of a block copolymer of poly(phenylene-ethynylene) (PPE) and poly(N-isopropylacrylamide) (polyNIPA) has been investigated by two synthetic routes: (1) PPE was synthesized with an end-cap containing atom transfer radical polymerization (ATRP) initiator, which was subsequently used as a macro-initiator for following ATRP of NIPA. This approach was not successful because of difficulties for incorporating ATRP initiators to functional PPE polymers. (2) PolyNIPA was polymerized using an initiator based upon iodophenyl derivatives, and thus functionalized block was used to endcap a PPEpolymerization. This approach gave a mixture of tri-, di-block and homo polymers of PPE and polyNIPA that showed fluorescence quenching at high temperatures resulting from the thermally induced aggregation by the polyNIPA.
5.1 Overview

5.1.1 Thermally responsive conjugated polymers

In the previous chapter, water-soluble conjugated polymers were developed for use in an aqueous environment in an effort to produce ultra-trace detection schemes that use an approach, which is different from conventional methods with conjugated polyelectrolytes. In this chapter, the application of water-soluble conjugated polymers is proposed wherein the optical properties in aqueous polymer solutions are controlled by thermally induced aggregation of poly(N-isopropylacrylamide) (polyNIPA)\(^1\) conjugated to the conjugated polymer. The design principle is depicted in Figure 5-1. Below the phase separation temperature, the copolymer is soluble in water, and thus the solution is fluorescent. By contrast, above the phase separation temperature, the copolymer aggregates with the collapsed polyNIPA, and the fluorescence is quenched. In this design, the fluorescence of PPE can be reversibly controlled by temperature.

A block copolymer\(^*\) was chosen as a platform because interactions between PPE polymer chains upon aggregation are necessary to change their optical properties. In the case of PPE-graft-polyNIPA\(^\circ\), such as that synthesized in Chapter 4, the collapsed

\* Block copolymers are liner polymers, which often consist of two or three different polymers connected with their polymer chain ends. When two different polymers are connected, the polymer is called di-block polymer. In the case of three polymers, it is tri-block copolymer.

\(\text{Di-block copolymer} \quad \text{Tri-block copolymer}\)

\(\circ\) PPE-graft-polyNIPA (Polymer C)
polyNIPA surrounds the PPE polymer chains at high temperatures and may isolate the PPE chain from each other, even in the aggregated state. This restricts the interactions necessary for quenching or energy transfer, both of which generally require a short distance between the chromophores/quenchers*. Therefore, block copolymers are expected to enhance the interaction between PPEs, and thereby change the fluorescence (quenching) in response to temperature.

\[ \text{Homogeneous solution} \quad \xrightleftharpoons{\text{Temperature}} \quad \text{Phase separation/aggregation} \]

* Fluorescence resonance energy transfer (FRET) is a distance-dependent dipole interaction of chromophores (a donor and an acceptor) through space. The energy of the donor is transferred to the acceptor without emission when the distance between them is in the range of 50-100 Å. Another requirement for FRET is an overlap of the donor emission and the acceptor absorption. In the FRET mechanism, when the donor is excited, the emission from the acceptor is observed.
5.1.2 Block copolymerizations

The two approaches were investigated:

(1) PPE macroinitiator: PPE was prepared with an end-capping group containing an ATRP initiator, which was designed to function as a macroinitiator for the ATRP synthesis of NIPA. In this approach, the molecular weight of polyNIPA is controlled by choosing the appropriate ATRP conditions (feed composition of monomers, reaction time). PolyNIPA only propagates from the PPE-macroinitiator, hence only tri-block copolymers would be made, facilitating the purification and separation of polymers.

(2) PolyNIPA end-capped PPE: PolyNIPA was polymerized using an initiator based upon iodophenyl derivatives by the ATRP method. PPE was the polymerized in the presence of this functionalized polyNIPA. The polyNIPA is then connected with the PPE through a cross-coupling reaction with the terminal iodophenyl group. This is a model synthesis that can be extended to include bio-conjugates of PPE that could be synthesized by polymerization of PPE in the presence of iodophenyl functionalized peptides or DNAs.
5.2 Results and discussion

5.2.1 PPE macroinitiator

ATRP initiator/end-cap 1 was added to a palladium catalyzed cross-coupling polymerization in an attempt to incorporate it into a hydrophilic PPE (Scheme 5-1). Monomers 6 and 7 were polymerized in morpholine by Pd-catalyzed cross-coupling reaction in the presence of 1, however it was found that the chloropropionate portion of 1 reacts with morpholine even at room temperature to give 8. To avoid nucleophilic reaction with secondary amines (morpholine), tertiary amines and other solvent systems were examined for the polymerization. However, only morpholine afforded the desired polymer (Table 5-1). Although there may be other ATRP initiators that are stable to the polymerization conditions, ATRP initiators generally have active halogen atoms that cannot survive cross-coupling conditions.
Scheme 5-1

Table 5-1. Polymerization conditions of PPE

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Amine</th>
<th>Polymerization</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Morpholine</td>
<td>Polymer (See Chapter 3 for the details)</td>
</tr>
<tr>
<td>DMF</td>
<td>DIPA</td>
<td>Crashed out during polymerization.</td>
</tr>
<tr>
<td>NMP</td>
<td>Et₃N</td>
<td>No polymer.</td>
</tr>
<tr>
<td>-</td>
<td>N-methylmorpholine</td>
<td>Monomers are insoluble. No polymer.</td>
</tr>
<tr>
<td>-</td>
<td>Et₃N</td>
<td>Monomers are insoluble. No polymer.</td>
</tr>
<tr>
<td>-</td>
<td>Hunig’s base</td>
<td>Monomers are insoluble. No polymer.</td>
</tr>
</tbody>
</table>

DIPA: diisopropylamine, Et₃N: triethylamine; Hunig’s base: N, N-diisopropylethylamine; NMP: N-methylpyrididione
To incorporate ATRP initiators into PPE, another synthetic route was examined using amine end-cap 2, following amide formation by reaction with the succinimide ester 5. PPE was synthesized in the presence of 2 instead of 1 (Scheme 5-1) to give an amine endcapped PPE, and a successive reaction of the amine with 5 was carried out in DMF. The amide formation was confirmed by the presence of $^1$H NMR signals associated with the methyl protons of 2-chloropropionic group at 1.5 ppm. An additional peak at 1.6 ppm was observed which may be the result of a side reaction of 5 with pendant hydroxyls to form an ester. ATRP of NIPA was carried out in the presence of the PPE macroinitiator and a CuCl/Me$_6$TREN catalyst in DMSO or DMF at room temperature. The resultant copolymer was not water-soluble, and the degree of polymerization of NIPA was 10 units based on $^1$H NMR analysis. The low degree of polymerization of NIPA may reflect a reduced grafting efficiency. Of primary concern is the fact that the Pd catalyst can modify the amine during the initial polymerization to form the PPE. Alternatively, there is the possibility that an $S_N$2 reaction of the amine with 2-chloropropionic group of 5 occurs. A model oligomeric compound, however, was successfully quantitatively modified with 5 and polyNIPA by ATRP. Another concern is that the Cu catalyst for ATRP might interact with PPE by chelating with the diethanol amide groups or the acetylenes, which would affect ATRP process.

![NIPA and Me$_6$TREN](image)

To eliminate undesired side reactions of the amine group on 2, end group containing protected amines$^2$ (t-BOC protected compound 3 and silyl carbamate
protected 4) were synthesized and examined. The protecting groups were chosen to avoid cross-reactions with the functional groups of the PPE. End-capping of PPE with 3 to generate PPE-BOC polymer instead of 1 (Scheme 5-1) was successful and incorporation was confirmed by \(^1\text{H}\) NMR analysis (\textit{tert}-butyl group of \(t\)-BOC). Unfortunately, the treatment of PPE-BOC with TFA caused a black colored solution, thereby indicating that the chemical structure of the conjugated polymer chain was changed by the acid. In general, strong acids react with the unsaturated bonds in PPEs and cause cross-links that result in black precipitates. Conjugated polymers are often stable to basic conditions, however, another conventional candidate of F-Moc\(^*\) is not stable to the PPE polymerization conditions because morpholine solvent was too basic.\(^2\) Another candidate was 4, in which the silyl carbamate could be cleaved by TBAF.\(^3\) This approach was expected to be a mild condition for the selective removal of this protecting group. The silyl carbamate of 4 was unfortunately not stable to the PPE polymerization conditions even at room temperature. In summary, a number of PPE-macroinitiator approaches including those using protecting groups presented difficulties for the incorporation of an ATRP initiator into the PPE. However, we were successful at the synthesis of macroinitiators for NIPA radical polymerization by a nitroxide initiator for nitoxide mediated radical polymerization (NMRP) as will be discussed in Chapter 6.

\* 9-Fluorenylmethyl carbamate

112
5.2.2 PolyNIPA end-capped PPE

5.2.3 Synthesis of block copolymer of PPE and polyNIPA

Given our problems with initiating a NIPA polymerization from a functionalized PPE as was just described, we endeavored to endcap a PPE polymerization with a functionalized polyNIPA. PolyNIPA was polymerized using initiator 1 by ATRP and CuCl/Tris(2-dimethylaminoethyl)amine (Me₆TREN) as the catalyst (Scheme 5-2). The polymerization was carried out in the conditions; monomer:solvent = 1:3 (wt/vol.), monomer:initiator:CuCl:Me₆TREN = 100:1:1:1. The polymer was precipitated in diethyl ether, dried, and the copper ion was removed by stirring with alumina in acetone. The Mn, Mw and polydispersity index (PDI) of the polyNIPA used here were 16,559, 28,812 and 1.72 respectively, based on a PMMA standard in GPC with DMF.

Scheme 5-2

Monomers 6 and 7 were polymerized using a Sonogashira coupling reaction in morpholine in the presence of polyNIPA 9 (Scheme 5-3). In Chapter 4, oligoNIPA (the degree of polymerization is 10-20) that was synthesized by the same ATRP conditions using initiator 1 was characterized by ¹H NMR. In this NMR analysis, the iodophenyl group as expected had the same ¹H NMR spectra before and after ATRP of NIPA,
thereby indicating that the iodophenyl group remains intact after the radical polymerization. The terminal iodophenyl group attached to the polyNIPA is designed to react with (endcap) the growing PPE chain. After polymerization, polymer 10 was purified by dialysis in water and the Mn, Mw and PDI were 20,440, 29,384, and 1.43, respectively (GPC, eluent:DMF, PMAA standard).

Scheme 5-3

The copolymer 10 is water soluble, and aqueous solutions of the copolymer were prepared by cooling a suspension of the polymer in an ice bath. The resulting solution was homogeneous and fluorescent at room temperature. PolyNIPA chains extend and swell at low temperature, which prevents the aggregation of polymers. Additionally, the polyNIPA enhances the water solubility, and the block copolymers are more soluble in water than the isolated PPE block. At high concentration, precipitation was observed
with heating as is to be expected from the aggregation of polyNIPA. The molecular weight of copolymer was lower than expected for a tri-block copolymer (polyNIPA-PPE-polyNIPA) when we consider the molecular weight of the starting polyNIPA. The copolymer provided was likely a mixture of a tri- and di-block copolymers as well as some homopolymers. The phase separation behavior could be useful for purification of copolymers in the future since, at high temperatures, only PPE block copolymers containing polyNIPA precipitate while PPE homopolymers remain in solution.

5.2.4 Spectroscopic characterization and temperature dependence

Figure 5-3 shows the fluorescence spectra of copolymer 10 in aqueous solution at various temperatures. Although the solution was diluted such that no precipitation was observed, even at high temperatures, the fluorescence intensity decreased with increasing temperature. As a control, we investigated a simple mixture of PPE and polyNIPA homopolymers, and we observed no significant temperature dependence when compared with the copolymer. Hence, the polyNIPA component of the copolymers is responsible for the thermally induced change in the fluorescence intensity. The intensities of the two distinct peaks at 425nm and 500nm, however, have different temperature dependencies. This suggests that there are at least two different polymer organization within the aggregates. Generally, aggregation of PPE produces a new energy emission feature around 500nm, and hence we believe the increased relative intensity in the long wavelength emission is diagnostic of aggregation.

In Figure 5-3 (b), the fluorescence intensity at 425nm is plotted as a function of temperature. The intensity decreases with increasing temperature and becomes constant
at high temperatures. Considering the phase separation temperature of polyNIPA aqueous solutions (32°C), the copolymer is fully solvated at the lower temperatures, but aggregates intermolecularly through the collapse of polyNIPA at higher temperatures. The temperature dependence of fluorescence shows a strong hysteresis with thermal cycling. This later effect is probably due to strong interactions between the polymer chains.

![Graphs showing fluorescence intensity vs. temperature and wavelength.](image)

**Figure 5.3.** (a) Fluorescence of PPE-polyNIPA copolymer 10 in aqueous solution at several temperatures ($\lambda_{ex} = 325$nm). The intensity decreased with increasing temperature. (b) Fluorescence intensity at 450nm is plotted as a function of temperatures.

### 5.3 Conclusion

The modification of the end groups of a hydrophilic PPE with an ATRP initiator in an effort to synthesize block copolymers with NIPA was not successful due to functional groups incompatibilities (hydroxyl and amide groups) with the PPE or its synthesis. Tsolakis *et al.* presented the synthesis of di- or tri-block copolymers containing oligo($p$-phenylene-ethynylene) as an initiator for ATRP and polystyrene.
However, the oligomer was simple as it is not water-soluble and has inert alkyl side chains and hydroxyl groups only at the polymer terminals. In this previous case, the oligomer has no functional groups other than the hydroxyl groups, which is simpler due to the lack of side reactions. In contrast, functional groups such as many hydroxyl and amide groups contained in the PPE used in this research severely restrict the chemistries and reaction conditions and a spectrum of problems interferes with sequential ATRP synthesis of polyNIPA.

PolyNIPA with an iodophenyl end-cap was prepared by ATRP and served as successful end-cap for PPE. The copolymers were simply obtained by polymerization of PPE in the presence of the polyNIPA functionalized with the iodophenyl group. It is likely that the resultant PPE/polyNIPA copolymer is a mixture of tri-, di-block and homopolymers. Despite the polymer mixture, the fluorescence of this copolymer is quenched at high temperatures because of the thermally induced aggregation of polyNIPA. These responsive polymers can be useful in the development of new biosensory materials and bio-conjugates.
5.4 Experimental procedures

5.4.1 General

Synthesis of compounds 1 and 2 was described in Chapter 4. Fluorescence spectra were obtained by a SPEX Fluorolog-c2 spectrofluorometer. The temperature of the solution was controlled by a folder equipped with a re-circulating chiller (RTE bath/circulator with microprocessor controller, NESLAB). NMR spectra were obtained on Varian Unity or Mercury 300 spectrometer. High-resolution mass spectra were obtained at the MIT Department of Chemistry Instrumentation Facility (DCIF) on a Bruker Daltronics APEX II 3 Tesla FT-ICR-MS.

5.4.2 ATRP initiator/end-cap groups

**Compound 3:**

\[
\begin{array}{c}
\text{H}_2\text{N} \rightarrow \text{O} \rightarrow \text{O} \rightarrow \text{NH}_2 \\
\text{(excess)}
\end{array}
\xrightarrow{\text{(BOC)O}}
\begin{array}{c}
\text{H}_2\text{N} \rightarrow \text{O} \rightarrow \text{O} \rightarrow \text{BOC} \\
\text{Di-I-butyl dicarboxylate (2.45g, 11.2mmol) in dioxane (30mL) was added by an addition funnel to dioxane solution (30mL) containing 2, 2'-}(\text{ethylenedioxy)}
\end{array}
\xrightarrow{\text{CH}_2\text{Cl}_2, \text{EtN}}
\begin{array}{c}
\text{H}_2\text{N} \rightarrow \text{O} \rightarrow \text{O} \rightarrow \text{BOC} \\
\text{Water (50mL) was added to the residue. The aqueous solution was extracted with CH}_2\text{Cl}_2 (50mL \times 3). The combined organic layer was back-washed with water (10mL \times 2) and dried over MgSO}_4. The solution was filtered and evaporated. The obtained oil was dried under vacuum. 4-Iodobenzoic chloride (1.2g, 4.8mmol) in CH}_2\text{Cl}_2 (5mL) was added to the crude product (Boc-protected amine, 1.0g, 4.0mmol) in CH}_2\text{Cl}_2 (5mL)/ \text{Et}_3\text{N}}
\end{array}
\]

Di-I-butyl dicarboxylate (2.45g, 11.2mmol) in dioxane (30mL) was added by an addition funnel to dioxane solution (30mL) containing 2, 2'-(ethylenedioxy) bis(ethylamine) (12.5g, 84.3mmol). After 3 hours, the solution became turbid and precipitated. The solution was kept stirred overnight, and the solvent was evaporated off. Water (50mL) was added to the residue. The aqueous solution was extracted with CH₂Cl₂ (50mL × 3). The combined organic layer was back-washed with water (10mL × 2) and dried over MgSO₄. The solution was filtered and evaporated. The obtained oil was dried under vacuum. 4-Iodobenzoic chloride (1.2g, 4.8mmol) in CH₂Cl₂ (5mL) was added to the crude product (Boc-protected amine, 1.0g, 4.0mmol) in CH₂Cl₂ (5mL)/ Et₃N
(1mL) by a syringe at 0°C. The reaction mixture was allowed to be at room temperature. The solution was diluted with CH₂Cl₂, washed with water and dried over MgSO₄. After filtration, the solvent was evaporated. The crude product was purified with silica gel chromatography (CH₂Cl₂ (95)/MeOH (5)). The obtained compound was solidified under vacuum (1.4g, 73% with respect to Boc-protected amine). ¹H NMR (300MHz, DMSO- d₆); δ 8.59 (t, 1H, J = 5.1Hz), 7.86 (d, 2H, J = 8.1Hz), 7.64 (d, 2H, J = 8.4Hz), 6.77 (t, 1H, J = 7.5Hz), 3.52 (m, 8H) 3.42 (m, 2H), 3.07 (q, 2H, J = 5.7Hz) 1.37 (s, 9H).

**Compound 4**:  

![Chemical structure](image)

2 (130mg, 0.344mmol) was placed in an oven dried 2-neck flask, and CH₂Cl₂ (3mL) and Et₃N (0.05mL) were added. Dry ice (300mg, 6.87mmol) was added to the flask under Ar. After 30 minutes, t-BuPh₂Si-Cl was added by a syringe. The reaction mixture was stirred at room temperature overnight and washed with water. The aqueous layer was extracted with CH₂Cl₂ and EtOAc. The combined organic layer was washed with sat. NaHCO₃ aq., sat. NaCl aq. and dried over MgSO₄. Evaporation after filtration gave an oily compound. This crude compound was purified by a silica gel chromatography (EtOAc (80)/hexane (20)), (130mg, 58%). ¹H NMR (300MHz, DMSO- d₆); δ 8.6 (t, 1H, J = 6Hz), 7.80 (d, 2H, J = 8.7Hz), 7.68 (m, 6H), 7.41 (m, 7H), 3.52 (m, 8H) 3.42 (m, 2H), 3.07 (m, 2H) 1.00 (s, 9H).
Compound 5:

\[
\begin{array}{c}
\text{Cl} \quad \text{Cl} \\
\text{O} \quad \text{O} \\
\text{N=O-H} \\
\text{CH}_2\text{Cl}_2, \text{Et}_3\text{N} \\
\rightarrow \\
\text{O} \quad \text{O} \\
\text{N=O-Cl} \\
\end{array}
\]

\(N\)-hydroxysuccinimide (1.00g, 8.69mmol) in dry \(\text{CH}_2\text{Cl}_2\) (37mL)/\(\text{Et}_3\text{N}\) (1.3mL) was cooled down on an ice bath. 2-chloropropionic chloride (1.16g, 9.12mmol) was added to the solution dropwise. The reaction mixture was allowed to room temperature and stirred for 8 hours. To this solution, ethylether (37mL) was poured, and the resultant precipitate was filtered off. The filtrate was washed with water, 10% \(\text{NaHCO}_3\) and dried over \(\text{MgSO}_4\). Evaporation of the solvent after filtration gave a red oily compound, which was solidified slowly in a refrigerator (1.73g, 92%). \(^1\text{H NMR}\) (300MHz, \(\text{CDCl}_3\)): \(\delta\) 4.690 (q, 1H, \(J = 6.9\text{Hz}\)), 2.863 (s, 4H), 1.867 (d, 3H, \(J = 6.9\text{Hz}\)).

5.4.3 Block copolymerization conditions

PolyNIPA 9 (256mg, 0.00136mmol of iodopheny group) and monomer 6 (40.0mg, 0.00613mmol) and 7 (30.6mg, 0.0681mmol) were placed in a 25mL Schlenk tube. \(\text{Pd}(\text{PPh}_3)_4\) and CuI were added to the tube in a glove box. Morpholine was purified by passing through an alumina column, and 1ml of morpholine was added to the tube. The reaction solution was heated at 50°C and stirred overnight. This solution was diluted with water and sat. \(\text{NH}_4\text{Cl}\) aq. solution and dialyzed against water. The resultant precipitate was collected by a filtration and dried under vacuum. \(\text{Mn}, \text{Mw}, \text{PDI}\) were 20,440, 29,384, and 1.43, respectively (GPC, eluent:DMF, PMAA standard).
5.4.4 Fluorescence measurements

The copolymer was dissolved in de-ionized water by cooling down in an ice bath for 10 minutes. The fluorescence cell containing the polymer solution was placed in a folder equipped with a flow circulation thermo-control system. The temperature on the folder was monitored. The fluorescence was measured 30 minutes after temperature was changed (about 5°C increase for each time). The excitation wavelength was 325nm.
Compound 3: $^1$H-NMR (300MHz, DMSO-$d_6$)

![NMR spectrum for Compound 3]

Compound 4: $^1$H-NMR (300MHz, DMSO-$d_6$)

![NMR spectrum for Compound 4]
Compound 5: $^1$H-NMR (300MHz, CDCl$_3$)
5.5 Reference

Chapter 6: Thermally responsive fluorescent conjugated polymers

Abstract: New thermally responsive block copolymers of water soluble fluorescent conjugated polymers and poly(N-isopropylacrylamide) have been synthesized that phase-separate from aqueous solutions upon heating. This phase separation behavior was demonstrated to thermally switch on fluorescence resonance energy transfer (FRET) between conjugated polymers and a fluorescent dye.
6.1 Overview

6.1.1 Thermally responsive conjugated polymers

In this chapter, it is demonstrated that the water-solubility of conjugated polymers can be thermally controlled using poly (N-isopropylacrylamide) (polyNIPA). New block copolymers of water-soluble conjugated polymers and polyNIPA, as shown in Chart 1 PPE-polyNIPA I, PPE(Me)-polyNIPA II and PPBT-polyNIPA III, are designed. As polyNIPA precipitates from water at temperatures >31°C by a dehydration mechanism, the copolymers phase-separate from aqueous solution. These block copolymers have been synthesized by making use of nitrooxide-mediated radical polymerization (NMRP) of NIPA from an initiator at the end of a precursor conjugated polymer. In the previous chapter, PPE-polyNIPA block copolymers were synthesized by polymerization of the PPE segment in the presence of end capping group that had been functionalized polyNIPA, and thermally induced fluorescence quenching of the copolymer in aqueous solution was observed. The block copolymers obtained here have a more precise structure as a result of an improved method for the synthesis of the NIPA block polymer. We have demonstrated the utility of the copolymers in fluorescence resonance energy transfer (FRET) from the polymers to a small dye molecule attached to polyNIPA.
6.1.2 Nitroxide-mediated radical polymerization (NMRP)

Considering the poor performance of the atom transfer radical polymerization (ATRP)* of NIPA reported in Chapter 4, we decided to attempt the synthesis of NIPA by NMRP. NMRP is an established method developed for the precision synthesis of polymers, providing a monodisperse molecular weight and functionalities for further modifications to block-, branched- and graft-structures.\(^2\) The NMRP-polymerizable monomers had been limited to less functionalized monomers (such as styrene or

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* ATRP is a metal-catalyzed radical polymerization where the equilibrium between radical and dormant (non-radical) forms is shifted to the dormant form, which eliminates termination processes involving radical-radical coupling because of a low radical concentration during polymerization.

127
acrylate), and lacked the substrate scope exhibited by ATRP until the recent development of new alkoxyamine initiators by Hawker and coworkers.\textsuperscript{3} The general NMRP mechanism for styrene is shown in Scheme 6-1.\textsuperscript{3} Alkoxyamine derivatives are used as initiators, and the weak C-O bond of the alkoxyamine thermally homolyzes to give a carbon radical and a nitroxide radical above about 100 °C. The carbon radical reacts with monomer (styrene), and the propagation of this radical reaction produces a polymer chain. During propagation, the polymer end (radical) is reversibly deactivated by coupling with nitroxide radical to give a dormant chain that can be reactivated by C-O bond breaking. This reversible reactivation/deactivation process of the radical species seems to control the polymerization, and the chains spend most of their time in the dormant form. This lowering of the carbon centered radical concentration reduces irreversible terminal processes due to radical-radical coupling. In this mechanism, polymers are synthesized in a controlled manner, thereby allowing a precise synthesis of well-defined polymeric architectures. We further note that the combination of the NMRP and ATRP offers a number of opportunities to create highly functionalized macromolecules.
Scheme 6-1

Initiation:

\[
\text{Initiator} \xrightarrow{\Delta} \text{Monomer} + \cdot\text{O}_{-N^R}^R
\]  \hspace{1cm} (1)

Propagation:

\[
\text{Polymer} \xrightarrow{K_{eq}} \text{Monomer} + \cdot\text{O}_{-N^R}^R \]  \hspace{1cm} (2)

The main contribution to \(K_{eq}\) is the reactivity of alkoxyamine initiator reflected by the structures of substituted groups (R and R') on the nitrogen. Therefore, their appropriate design is critical for this controlled polymerization. Case studies for monomers have been carried out to find suitable nitroxide structures, and the development of universal initiators has been pursued. Harth et al. reported NMRP of NIPA and \(N,N\)-dimethylacrylamide by using alkoxyamine initiator 1, and they obtained polymers with accurately controlled molecular weights and low polydispersity. They also succeeded in the synthesis of a block copolymer of polyNIPA and polystyrene. In contrast, the polymerization of \(N,N\)-dimethylacrylamide using AIBN as an initiator and 2,2,6,6-tetramethyl-1-piperidinyloxyl nitrooxide (TEMPO) did not display "living"
character.\textsuperscript{5} The structure of the nitroxide groups (alkoxyamines) and their reactivity are clearly important factors for the “living” nature of radical polymerization processes.\textsuperscript{3,4}

![ TEMPO and nitroxide structure ]

6.2 Results and discussion

6.2.1 Synthesis of PPE-polyNIPA block copolymers

The end-cap/NMRP initiator 3 was prepared from the ATRP initiator 2 that was described in Chapter 4 by adapting methods developed by Matyjaszewski (Scheme 6-2).\textsuperscript{6} The ATRP initiator produces a radical upon reaction with CuCl, and the radical can then be captured by the nitroxide radical (TEMPO or di-\textit{t}-butyl nitroxide) or the CuCl\textsubscript{2}/Me\textsubscript{3}TREN to give back 2. Addition of Cu (0) favors the formation of 3 by reducing the Cu(II) splices that serve to regenerate 2, thereby allowing the reaction to reach completion.

Scheme 6-2

![ Scheme 6-2 ]

The water soluble poly(phenylene-ethynylene) derivative (PPE) 6 in Scheme 6-3 was synthesized from monomers 4, 5 and the end-capping/NMRP initiator 2 by a Sonogashira cross-coupling using a Pd catalyst and CuI in morpholine, and was purified
by precipitation and dialysis against water. The details of the polymerization of watersoluble PPEs were described in Chapter 3, and we have recently published an initial account of this process.\textsuperscript{7} NMR analysis provided determination of the degree of polymerization (DP) by integrating the signals from aromatic and amide groups in the PPE relative to from the tert-butyl moieties of the endgroup that are well-resolved from other polymer signals. The DP of 6 was found to be 11.5. In this reaction scheme, molecular weights of 6 can be controlled by feed compositions of monomers and initiators.\textsuperscript{8}

Scheme 6-3

Conditions: a) 2, Pd(PPh\(_3\))\(_4\), Cul, morpholine, 60\(^\circ\)C, b) NIPA, DMF or DMSO, 120\(^\circ\)C
NMRP of NIPA was carried out using 6 as an initiator in DMF or DMSO at 120°C. The polymers were purified by precipitation into diethyl ether and dialysis against water. In contrast, NMRP of NIPA using a TEMPO-derivative initiator gave no polymer. Generally, the di-tert-butyl nitroxide initiator has higher reactivity than TEMPO derivative initiators because the steric hindrance from the tert-butyl groups weakens the C-O bond, thereby lowering the temperatures required for polymerization. NMRP of NIPA using only initiator 2 without the PPE segment was less controlled; the DP was calculated to be 38-46 based on 1H NMR analysis although the expected DP was 100, and the yield was 14%.

Tsolakis et al. recently reported a synthesis of block copolymers of an oligo(p-phenylene-ethynylene), which initiating an ATRP of styrene from the polymer chain ends. In the beginning of this investigation, we also attempted incorporation of an ATRP initiator into the terminal group of a PPE using the same procedures above. However, the chloropropionic acid derivative, which was used as an initiator for ATRP of polyNIPA (Chapter 4), was not stable to the Sonogashira coupling conditions (described in Chapter 5). In the contrast, the nitrooxide derivative 2 was stable to PPE polymerization conditions and is an effective initiator in the NMRP of NIPA.

Stalmach et al. reported the synthesis of di-block copolymers of poly(phenylene-vinylene) (PPV) and poly n-butylacrylate (PBA) (PPV-b-PBA) by NMRP and observed microphase-separated morphology in transmission electron microscopy studies. The PPV with a narrow polydispersity in its molecular weight was prepared first, and then one end of this material was modified by a nitroxide initiator group for subsequent NMRP of PBA. In our case, the molecular weight distribution of PPE has a
polydispersity close to 2.0 because the cross-coupling polymerization is subject to the chain growth statistics of a step-growth mechanism. Our initial goal is only to thermally induced phase separation of conjugated polymers, therefore we do not require precise polymer morphologies and regulated structures. Hence in our approach, the polydispersity of PPE is acceptable, and the average molecular weight can be controlled by the feed composition of the monomers and the end caps. Once the doubly endcapped PPEs are in hand, a one-step modification can then be used to create tri-block copolymers of conjugated polymers and other vinyl polymers.

The UV-vis absorption spectra of PPE-polyNIPA I and the precursor PPE 6 are shown in Figure 6-1. After copolymerization, a blue shift in PPE-polyNIPA I’s absorption spectra was observed, whereas the fluorescence showed no shift. We can eliminate the possibility that the shorter wavelength UV-vis absorption peak is due to the fact that higher molecular weights are eliminated by the NMRP process, because in that case, the fluorescence peak should also be blue-shifted, which is not observed in Figure 6-1. A more plausible explanation is that the copolymerized polyNIPA disturbs the conjugation of the PPE through steric hindrance, which creates a higher energy gap in the electronic structure of the PPE and a commensurate blue-shift in UV-vis absorption spectrum. The lack of change in the fluorescence is likely due to energy migration. The excited states migrate through the PPE polymer chain to a lower energy band gap that is expected at the center portion of the PPE segments, which is less affected from polyNIPA. In this way, the emission corresponding to the lower energy gap dominates and the spectrum appears to be the same as that of 6.
Figure 6-1. UV-vis absorption and fluorescence spectra of PPE 6 (solid lines with circles and with squares) and PPE-polyNIPA I (broken lines with circles and with squares). The excitation wavelengths are 380nm for PPE and 350nm for PPE-polyNIPA I.

PPE (Me)-polyNIPA was synthesized by the same procedure as PPE-polyNIPA I using dimethoxydiethynylbenzene instead of monomer 5. PPE(Me)-polyNIPA II is readily soluble in water. As shown in Figure 6-2, PPE(Me)-polyNIPA II displays additional longer wavelength peaks in its absorption and emission relative to that of PPE-polyNIPA I shown in Figure 6-1. These additional features are characteristic of aggregated conjugated polymers that exhibit a new low-energy band gap due to strong electronic interaction between their conjugated backbones. This result indicates that the aggregation of PPE(Me) is sufficiently strong that the polymers remain associated despite the polyNIPA and its high solubility in water. In comparison, the chemical structure of PPE-polyNIPA I has greater hydrophilic bulk surrounding the polymer backbone that provides more hydration, which prevents polymer aggregation and promotes superior water solubility.
Figure 6-2. UV-vis absorption (dashed line) and fluorescence (solid line) spectra of PPE(Me)-polyNIPA II in aqueous solution. The excitation wavelength was 350 nm.

6.2.2 Poly(phenylene-bithiophene) (PPBT)

A poly(phenylene-bithiophene) (PPBT) derivative 8 was prepared from the monomers 4 and 7 by a Pd catalyzed Stille coupling reaction\textsuperscript{11} in morpholine in the presence of 2 as an endcapping group (Scheme 6-4). The polymer was purified by precipitation and dialysis against water. A water insoluble oily dark material formed in the dialysis tube was filtered off, and the filtrate was lyophilized. The discarded oily material is likely organotin compounds that are a side product of the synthesis. The purified polymer is readily soluble in water, and the DP of 30 was determined based on \textsuperscript{1}H NMR spectrum. NMRP of NIPA initiated from the terminal units of 8 produces PPBT-polyNIPA III. This block copolymer was further purified by dialysis against water, and the solution was lyophilized after filtration. The polymer was readily soluble in water and is weakly fluorescent in aqueous solution.
Scheme 6-4

Conditions: a) 2, Pd(PPh₃)₄, morpholine, 80°C, b) NIPA, DMF or DMSO, 120°C

The UV-vis absorption and fluorescence spectra of PPBT-polyNIPA III and PPBT 8 are shown in Figure 6-3. In contrast to the case of PPE-polyNIPA I, the absorption spectrum showed a red shift after the NMRP synthesis of the block copolymer. The cause of the red shift after NMRP is not clear. It is possible that molecular weight of PPBT in the block copolymer might be larger than the precursor PPBT as a result of the purification procedures. A higher molecular weight polymer is expected to have greater conjugation and a lower energy emission. The hydrophobic environment created by polyNIPA around PPBT may also induce longer π-conjugation in the polymer because it favors the hydrophobic thiophene backbone. In Figure 6-3, the PPBT-polyNIPA III emission peak position is further found to depend on the excitation
wavelength. In contrast, PPBT 8 showed only slight shifts in the emission spectra for different excitation wavelengths. This indicates that PPBT-polyNIPA III contains multiple emissive states. The hydrophobic environment of polyNIPA may be responsible for this behavior by affecting the electronic structure of the PPBT segments in the block copolymers and/or by inducing polymer aggregation.

![Graph](image)

**Figure 6-3.** UV-vis absorption and fluorescence spectra of PPBT 8 and PPBT-polyNIPA III

### 6.2.3 Thermally induced precipitation

We have been interested to develop sensor assays that make use of the fact that the PPE-polyNIPA I block copolymers can be phase-separated from aqueous solution upon heating. In this section, we describe the development of a scheme whereby polymers are collected as solids to facilitate analysis. To prepare solutions, the polymers were stirred in an ice bath. The swelling of polyNIPA chain at lower temperature dissociates the physical interpolymer associations caused by the lyophilizing process and
encourages the block copolymers to dissolve in water. Fluorescent assays will need to be performed at concentrations lower than is typically used in the precipitation of polyNIPA, and in order to cause quantitative phase separation, aqueous NaCl was added to the polymer solution. This polymer solution (polymer concentrations 0.25-0.025mg/mL) was heated at 50°C, and the resultant precipitate was collected by vacuum-filtration with a small filter paper (diameter 8mm) on a Hirsch funnel and washed with hot water to remove the salts. The filtrate had a yellow color and blue fluorescence. It appears that only trace amounts of the polymer passed though the filter paper. After drying under vacuum at room temperature, the filter paper was placed between a cover slip and a glass microscope slide, and the fluorescence from this assembly was measured. The studies we performed suggested that the emission spectra from the polymer precipitates samples on a filter paper indicated a reproducibility of the emission intensities was within 20%, which gave us useful data analysis to quantify the features of the optical properties of the polymer precipitates.

Figure 6-4 shows the fluorescence spectra of PPE-polyNIPA I on a filter paper. PPE-polyNIPA I on a filter paper exhibits only a slight red shift in the emission relative to that in solution, and no new emission signals were observed. Aggregation of conjugated polymers is generally enhanced in the solid state because of π-π stacking of the polymer chains, leading to strong electronic coupling. This often generates a new low energy band and undesired non-emissive aggregates. Hence the absence of such features indicates that the polymer chains are largely prevented for having strong interchain electronic interactions. In contrast to PPE-polyNIPA I, the block copolymer with less hydrophilic side chains Polymer 10 shown below (discussed in the previous chapter) is
quenched upon heating. The dendritic side chains of PPE-polyNIPA I likely discourage the polymer aggregation even in solid state and prevent the formation of non-emissive aggregates. In the case of PPBT-polyNIPA III, the polymer precipitate showed a red shift and lower fluorescence intensity (Figure 6-5), consistent with aggregation induced electronic interactions between the polymer backbones.

Figure 6-4. Fluorescence spectra of PPE-polyNIPA I in solution (solid line) and solid on a filter paper (broken line). The excitation wavelength is 350nm.
Figure 6-5. Fluorescence spectra of PPBT-polyNIPA III in solution (solid line) and solid on a filter paper (broken line). The excitation wavelength is 430nm.

6.2.4 Concentration dependence

The fluorescence intensity was measured in various PPE-polyNIPA I concentrations (Figure 6-6). The emission intensity from the polymers on the filter papers increased linearly with increasing polymer concentrations without the appearance of any new emission peaks. These results indicate that strong aggregation or dramatic conformational changes are not induced by precipitation. Furthermore, it confirms that our collection scheme using a filter paper accurately reflects the polymer concentration in solution. An apparent slight red shift of maximum intensity with increasing polymer concentration could be due to the background scattering from the filter paper.
Figure 6-6. Fluorescence spectra of PPE-polyNIPA I on filter papers (a) and intensities at 470nm (b). The intensity increased with increasing the polymer concentration (0.015, 0.03, 0.075 and 0.15mg/mL). The precipitates from 1mL of the polymer solutions were collected by a filter paper. The excitation wavelength was 350nm.

6.2.5 Fluorescence resonance energy transfer (FRET)

Fluorescence resonance energy transfer (FRET) is widely used in biological applications for the trace detection of biomolecules or analytes. FRET is a distance-dependent through space dipole interaction of chromophores (a donor and an acceptor). The energy of the donor is transferred to the acceptor without emission when the distance separating them is in the range of 50-100 Å, and thus FRET can be switched on or off by controlling the distance between the donor and acceptor. Another requirement for FRET is an overlap of the donor’s emission and the acceptor’s absorption. Pairs of chromophores should be designed and chosen to have a large overlap to encourage efficient FRET.

The detailed explanation on FRET mechanism was described below. The rate of the energy transfer $k_{ET}$ as analytically described by Förster as is as follows:
\[ k_{ET} \propto \left( \frac{1}{\tau_D} \right) \left( \frac{R_0}{R} \right)^6 \]  

(1)

where \( \tau_D \) is lifetime of the donor in the absence of the acceptor, \( R \) is the distance between the donor and the acceptor and \( R_0 \) is the Förster radius defined by the critical separation of the donor and the acceptor at which the emission from the donor and energy transfer are equally probable (50% efficiency of FRET).

\[ R_0 = \left[ 9.79 \times 10^3 \kappa^2 n^{-4} Q_D J(\lambda) \right]^{\frac{1}{6}} \]  

(2)

where \( \kappa^2 \) is dipole orientation factor given by

\[
\kappa^2 = \left[ \frac{\mathbf{E}_D \cdot \mathbf{A}_A - 3(\mathbf{E}_D \cdot \mathbf{R})(\mathbf{A}_A \cdot \mathbf{R})}{R^2} \right]^2 \\
= \left[ \cos \theta_T - 3 \cos \theta_D \cdot \cos \theta_A \right]^2 \\
= \left[ \sin \theta_D \cdot \sin \theta_A \cdot \cos \varphi - 2 \cos \theta_D \cdot \cos \theta_A \right]^2
\]  

(3)

![Diagram](image)

\( \mathbf{E}_D \) transition dipole of the donor  
\( \mathbf{A}_A \) transition dipole of the acceptor

**Figure 6-7.** Transition dipoles of donor and acceptor

\( \kappa^2 \) value is 4 for parallel transition dipoles (\( E_D \parallel A_A \)), 2/3 for randomly orientated transition dipoles and 0 for perpendicular transition dipoles (\( E_D \perp A_A \)). \( n \) is the refractive index, \( Q_D \) is the fluorescence quantum yield of the donor in the absence of the acceptor and \( J(\lambda) \) is the spectral overlap integral of donor's emission and acceptor's absorption.
\[ J(\lambda) = \int \varepsilon(\lambda) \cdot F_D(\lambda) \lambda^4 d\lambda \] (4)

**Figure 6-8.** The spectral overlap integral \( J(\lambda) \) in the FRET mechanism

where \( \lambda \) is the wavelength, \( \varepsilon(\lambda) \) is excitation coefficient of the acceptor and \( F_D(\lambda) \) is the fluorescence emission intensity of donor as a fraction of the total integrated intensity.

Hence, \( k_{ET} \) is dependent on many factors including the spectral overlap, orientation of the transition dipoles, lifetime, refractive index of the medium and the distance between the donor and the acceptor. Among them, the two primary factors most often modulated in FRET schemes are:

1. The distance between the donor and the acceptor, \( R \): \( k_{ET} \propto R^6 \) and hence the FRET is effective when \( R < R_0 \) where energy transfer dominates.
2. Spectral overlap integral \( J(\lambda) \): \( J(\lambda) \) value is larger as the spectral overlap is larger, which gives higher FRET efficiency.

### 6.2.6 Thermally induced FRET

It has been found that conjugated polymers have the ability to harvest light and transfer it efficiently to a small dye molecule (acceptors).\textsuperscript{12} The acceptor emission can be enhanced for a given photon flux because it can receive energy that has been harvested
by multiple polymer segments that are not proximate to it initially. The transport of the excited states (excitons) can bring the energy within the Förster radius \( R_o \), allowing capture by the acceptor. In this way, photon harvesting and energy migration by a conjugated polymer can enhance the emissive signal emitted from the acceptor.

To examine these principles, FRET between a PPE-polyNIPA I block copolymer and a small dye molecule, Rhodamine B (RhB) bound to polyNIPA (RhB-polyNIPA IV) shown below, was examined. RhB was chosen as a FRET partner for PPE-polyNIPA I because its fluorescence spectrum is well-resolved from that of PPE-polyNIPA I, making signal amplification quantifiable. Fluorescein is also a candidate as a FRET partner with PPE and has the advantage of a larger spectral overlap than RhB. However, the fluorescence of fluorescein conjugated to polyNIPA is dramatically diminished with aggregation of polyNIPA upon heating. This loss of emission from fluorescein is likely the result of the hydrophobic environment in the polyNIPA aggregates which shifts its equilibrium shown in Figure 6-9 from its fluorescent form to the colorless and non-fluorescent lactone form.

![RhB-PolyNIPA IV](image-url)
Taking advantage of the thermal responsive properties of PolyNIPA, thermal precipitation/aggregation of the polymers is expected to bring the PPE and RhB within the Förster radius ($R_0$) of each other to produce efficient FRET. In this way, a signal from the RhB will be amplified by FRET from PPE (Figure 6-10).

Figure 6-10. Schematic representation of thermally induced FRET mechanism
6.2.7 Optical properties of the block polymers

Normalized UV-vis absorbance and fluorescence spectra of PPE-polyNIPA I, PPBT-polyNIPA III and RhB-polyNIPA IV in water are shown in Figure 6-11. As can be seen by inspection, there are varying degrees of spectral overlap between pairs of donors (PPE-polyNIPA I and PPBT-polyNIPA III) and acceptors (RhB-polyNIPA IV). In the Forster mechanism, larger spectral overlap provides higher FRET efficiency (section 6.2.5). The PPBT-polyNIPA III emission spectrum has a larger overlap with RhB-polyNIPA IV absorption spectrum than that exhibited between the PPE-polyNIPA I and RhB-polyNIPA IV. In the absence of other interfering features, we expect this spectral overlap term to produce superior FRET.

![Figure 6-11](image)

Figure 6-11. Normalized UV-vis absorption and fluorescence spectra of PPE-polyNIPA I, PPBT-polyNIPA III and RhB-polyNIPA IV in water. The excitation wavelengths for B, D and F are 350, 440, and 350 nm respectively.
6.2.8 FRET between PPE-polyNIPA I and RhB-polyNIPA IV

The absorption and fluorescence spectra from mixed solutions of PPE-polyNIPA I and RhB-polyNIPA IV are shown in Figure 6-12 and Figure 6-13, respectively. When the solution was excited at 350nm, a major emission peak from PPE-polyNIPA I and a small one from the RhB bound to RhB-polyNIPA IV were observed at 470nm and 580nm, respectively. With excitation at 500nm, the RhB was selectively excited, and its emission was observed at 580nm. The fluorescence intensity from RhB in solution at both excitation wavelengths was found to be directly proportional to the RhB-polyNIPA IV concentration (Figure 6-13 c). The ability to directly excite the RhB allows this signal to be used as an internal reference.
Figure 6.12. Absorption spectra of solution mixtures of PPE-polyNIPA I and RhB-polyNIPA IV. PPE-polyNIPA I concentration was constant at 0.12mg/mL. RhB-polyNIPA IV concentrations are 0.05, 0.025, 0.0125, 0.00625mg/mL, and the absorbance at 560nm increased linearly with increasing RhB-polyNIPA IV concentration.

Figure 6.13. Fluorescence spectra of the mixture of PPE-polyNIPA I and RhB-polyNIPA IV in a solution (a), and on a filter paper (b). The spectra in (b) were normalized relative to the peak intensities at 468nm. PPE-polyNIPA I concentration in the solutions was 0.12mg/mL. RhB-polyNIPA IV concentrations are 0.05, 0.025, 0.0125 and 0.00625mg/mL in the solutions. PPE-polyNIPA I is the principle absorbing group with 350nm excitation, and RhB-polyNIPA IV is selectively excited with illumination at 500nm. Light scattering from the filter paper gives a peak at 540 nm. Fluorescence intensity at 580 nm increased with higher RhB-polyNIPA IV concentrations. The solid lines in (c) and (d) show relative dependence.
The polymers were thermally aggregated/precipitated and then collected on a non-fluorescent filter paper using procedures described earlier in this chapter. The normalized fluorescence spectra from the filter paper are shown in Figure 6-13, b. The original spectra of the polymer precipitates showed about 20% differences in the magnitude of the peak intensities at 468nm, which are emissions from the PPE segments. We often observed that the emission spectra from pure PPE-polyNIPA I precipitate samples on filter papers exhibited maximum 20% differences in the magnitude of emission intensities, which is regarded as the accuracy of our measurements including collecting procedures and fluorescence measurements. Moreover, we also confirmed that an addition of pure polyNIPA to PPE-polyNIPA I solutions does not affect the emission intensities from the PPE segments in the precipitates. This indicates that the 20% difference in the PPE emission intensities from the precipitates is not due to the presence of polyNIPA (RhB-polyNIPA IV), but the data scatter caused by the experimental procedures. In order to correct this experimental uncertainty and quantify the data analysis for FRET, we normalized the emission spectra from the polymer precipitates relative to the intensity values of PPE emission peaks at 468nm.

We are most interested in determining if the amount of energy transfer from the PPE to the RhB is increased in the precipitate relative to solutions. To this end, we find that the emission at 580nm from RhB is 5 times larger when we selectively excited the PPE at 350nm than when we selectively excited at 500nm. This effect is not due to the excitation intensity because the photon fluxes with excitation at 350 and 500nm are corrected to be the same by the spectrometer. The RhB was excited at 500nm rather than its absorption maximum to avoid an overlap with strong light-scattering from the filter
paper. The RhB absorbance maximum is at 560nm, and based upon the differences in excitation coefficient at 500nm, we would expect the emission to be 4 times larger than that obtained with 500nm excitation, which is 20% less than the RhB emission induced by FRET from the PPE. As a control, we find that there is no enhancement in the fluorescence in the pure RhB-polyNIPA IV precipitate. Therefore, the increasing signal from RhB with excitation at 350nm is due to FRET, which is induced by thermal precipitation. The linear dependence on RhB-polyNIPA concentration in RhB fluorescence intensity in solution is consistent with a lack of FRET when the donors and acceptors are not precipitates. Interestingly, the precipitates behave differently with the RhB fluorescence intensity at 580nm from the filter papers ($\lambda_{\text{ex}} = 350\text{nm}$) slightly leveling off with increasing RhB-polyNIPA IV concentration (Figure 6-13, d). This is not an issue of quantum yield as the intensity of RhB ($\lambda_{\text{ex}} = 500\text{nm}$) increased linearly in the precipitate with increasing RhB-polyNIPA concentrations. The origin of the leveling-off of RhB emission with 350nm excitation is an additional signature of energy transfer. At high RhB concentration, there are multiple RhB acceptors within the Förster radius and exciton diffusion of an individual PPE chain. In this way, the amount of energy available to each RhB acceptor from the PPE donors decreases.

In spite of our successful demonstration of energy transfer from PPE-polyNIPA to RhB-polyNIPA, there are a number of features that indicate that their process does not have high efficiency. First, we are only able to increase the RhB emission about 20% beyond that which can be obtained by direct excitation of this dye. Secondly, the donor (PPE) emission does not decrease significantly with increasing RhB concentrations, which indicates that only a small amount of the donor’s energy is being captured by the
acceptor. We have considered that the inefficiency in energy transfer from the PPE segments can be attributed to one or more of the following issues:

1. Inhomogeneous phase separation: The polymers have different phase separation temperatures resulting in the polymers precipitating separately.

2. Too large separation of the donor (PPE) and acceptor (RhB): If the RhB is imbedded in polyNIPA aggregates and located too far from the PPE, then FRET is less efficient.

3. Spectral overlap: Larger overlap of donor’s emission and acceptor’s absorption generally results in more efficient FRET. The spectral overlap of PPE and RhB may not be sufficient (Figure 6-11).

4. Low quantum yield of PPE-polyNIPA I: The PPE has a relatively low quantum yield, which could be attributed to a weak aggregation of the polymers. The aggregation of PPE thereby reduces the efficiency of FRET to the acceptor dye.

6.2.9 PPE-RhB-polyNIPA IV

In order to examine if FRET could be enhanced by placing the RhB in the same polymer, RhB was incorporated into the polyNIPA chain of PPE-polyNIPA I block copolymer to give PPE-RhB-polyNIPA V. The distance between RhB and PPE is expected to be reduced by placing both donor and acceptor in the same copolymer, and FRET efficiency can be further increased with simultaneous precipitation of both donor and acceptor, which must occur when they are covalently linked together. This copolymer was prepared by random copolymerization of NIPA and RhB-monomer using
6 as a NMRP initiator. The polymer was phase-separated by heating to 50°C and collected on a filter paper. The ratio of the absorption peaks of PPE (380nm) and RhB (570nm) is 10 (Figure 6-14), which is similar to the ratio displayed in the mixture with the highest concentration of PPE-polyNIPA I (0.12mg/mL) relative to RhB-polyNIPA IV (0.025mg/mL) that was shown in Figure 6-12. The fluorescence spectra from the solution and the thermal precipitates collected on a filter paper are shown in Figure 6-15. The RhB emission from the filter paper (\(\lambda_{ex} = 350\)nm) increased from that of the solution relative to the RhB emission with excitation at 500nm. Additionally, we now observed a pronounced decrease in the PPE emission relative to RhB emission. The intensity ratio of the RhB peak excited at 350nm relative to that excited at 500nm is 5, which is almost same order of enhancement as the mixture of PPE-polyNIPA I and RhB-polyNIPA IV in Figure 6-13. However, the decrease of intensity of the PPE emission at 470nm relative to the RhB (\(\lambda_{ex} = 500\)nm) suggests that the energy transfer may be more efficient in the single polymer precipitate than in the mixture precipitate of the polymers discussed in section 6.2.8. These results indicate that low spectral overlap of the PPE and RhB might be the limiting factor in FRET, rather than the distance between donor and acceptor.
Figure 6-14. Absorption spectrum of PPE-RhB-polyNIPA V in water

Figure 6-15. Fluorescence spectra of PPE-RhB-polyNIPA V solution (a) and a filter paper (b). The emission at 540 nm in the spectra (excitation at 500nm)(b) is a light scattering from the filter paper.

6.2.10 FRET between PPBT-polyNIPA III and RhB-polyNIPA IV

In order to improve the FRET efficiency with RhB, we have made investigation with the lower-bandgap emissive conjugated polymer, poly(phenylene-bithiophene) derivative (PPBT) 8 (DP = 34 based on the integration in $^1$H NMR spectrum) and FRET between PPBT-polyNIPA III and RhB-polyNIPA IV. PPBT-polyNIPA III has a larger emission overlap area with the RhB absorption (Figure 6-11) than that displayed by PPE,
which is more suitable for efficient FRET. The UV-vis absorption spectra of the mixture with different RhB-polyNIPA IV concentrations are shown in Figure 6-16, and a dominant absorption from PPBT at 430nm is observed. A mixed solution of the PPBT-polyNIPA III and RhB-polyNIPA IV was heated to 50°C to give a precipitate that was collected on a filter paper and dried under vacuum. The fluorescence from the filter paper showed a broad red shifted peak relative to that observed in solution. The aggregated form of PPBT-polyNIPA III appears to dominate the emission of the precipitate on the filter paper. We observed a decrease of PPBT emission intensity at 540nm with increasing RhB-polyNIPA IV concentrations relative to the RhB emission at 580nm ($\lambda_{ex} = 440$nm), indicating FRET from PPBT to RhB. However, we did not observe a dramatic increase of the RhB emission when PPBT was excited at 440nm relative to that when the sample was excited at 500nm, which is a location where RhB is the principle absorber. Additionally, PPBT and RhB emissions and absorptions are not well resolved, which limits the quantitative analysis of FRET like which is shown in Figure 6-13. The low efficiency of FRET in this case further illustrates the difficulties encountered with polymeric chromophores. The problems are likely due to the formation of weakly emissive PPBT-polyNIPA III aggregates that reduce the amount of energy transferred to RhB.
Figure 6-16. UV-vis absorption spectra of mixtures of PPBT-polyNIPA III and RhB-polyNIPA IV in aqueous solution. The concentrations of RhB-polyNIPA IV are 0.05mg/mL (a solid line) and 0.0063mg/mL (a dashed line).

Figure 6-17. Fluorescence spectra of mixtures of PPBT-polyNIPA III and RhB-polyNIPA IV on a filter paper. The excitation wavelengths are 440nm (a) and 500nm (b). The concentrations of RhB-polyNIPA IV are 0.0063 (solid lines), 0.0125 (solid lines with circles), 0.025 (solid lines with squares) and 0.05 mg/mL (triangles).
6.3 Conclusions

New thermally responsive conjugated polymers conjugated with polyNIPA have been synthesized. These copolymers phase-separate from aqueous solution upon heating, and we developed a quantitative assay of the resultant polymer precipitates by simple filtration and characterized by fluorescence spectroscopy. This thermal precipitation was utilized to enhance FRET between conjugated polymers and a fluorescent dye. PPE-polyNIPA I showed energy transfer to RhB bound to polyNIPA by thermal precipitation/aggregation. We also examined FRET schemes between PPBT-polyNIPA III and RhB-polyNIPA IV. FRET from PPBT-polyNIPA III to the RhB in the aggregates that thermally precipitated from the solution mixtures was not efficient, and this is likely due to the low quantum yield of PPBT that reduces its emission and restricts FRET efficiency. The development of highly emissive conjugated polymer aggregates and their copolymers with polyNIPA is required for more efficient FRET.

In closing this chapter, the potential applications of thermally responsive conjugated polymers are discussed. The potential of this polymer system is in the use of thermally induced separation with signal amplification by photo-harvesting FRET from the fluorescent conjugated polymers. Monji et al. have developed immuno separation and immunoassays using antibody-conjugates of polyNIPA, which precipitate from water above 31°C and can be re-dissolved by cooling. In their sandwich immunoassays, the antibody-conjugate of polyNIPA was incubated with an antigen in a solution containing a second florescent dye-labeled antibody. These two antibodies bind different epitopes of the antigen and thereby form an immuno sandwich complex. This bound immuno complex to polyNIPA could be precipitated from the solution by heating and
concentrated by centrifugation. After multiple washings to completely remove the unbound antibody, the polymer was re-dissolved in a small quantity of water by cooling, and the amount of the labeled antibody (immuno complex) was determined by measuring the fluorescence.

Using these thermal precipitation principles, we envision a new assay method using thermally responsive conjugated polymers (Figure 6-18). In this scheme, a solution containing a targeted antigen is heated with its antibody that is labeled a fluorescent dye and an additional antibody-conjugate of polyNIPA. These two antibodies are chosen to bind to different epitopes of the targeted antigen. Addition of these reagents to the antigen solution followed by incubation will give a sandwich immunocomplex. After addition of a thermally responsive PPE block copolymer, the solution is heated and the resultant precipitate containing immunocomplex and PPE can be separated by filtration using a filter paper. Generally, the amount of added labeled antibody is in excess to the antigen because the amount of the targeted antigen is unknown. In the filtration step, the unbound excess labeled antibody and other reagents can be removed, thereby removing background interference to the fluorescence measurements. The fluorescent signal from the dye attached to the antibody can then be detected by PPE amplified FRET. This thermal precipitation assay has advantages:

- Efficient interactions between reagents with rapid kinetics in solution
- Quick separation of a targeted antigen and washing
- Signal amplifications by photo-harvesting FRET

This assay principle can be potentially applied broadly to biosensing.
While the polymers generated here lack the performance to produce a reliable assay of this type, we have established the method principle for the development of this assay. Our ongoing efforts in the Swager group are directed at solving the problems encountered, which include low water solubility, self-quenching by aggregation and non-optimal spectral overlap.

6.4 Acknowledgements

I thank Ms. Phoebe Kwan for providing compound 6 (5, 5'-bis(trimethylstanny)-2,2'-bithiophene).
6.5 Experimental procedures

6.5.1 General

Synthesis of the monomers 4, 5 and ATRP initiator 2 was described in Chapters 4 and 5, respectively. Fluorescence spectra were measured on a SPEX Fluorolog-t2 spectrofluorometer. CuCl was purified by stirring in acetic acid overnight. The remaining solid was filtered, washed with EtOH, dried under vacuum and stored under Ar. NMR spectra were obtained on Varian Unity or Mercury 300 spectrometer. High-resolution mass spectra were obtained at the MIT Department of Chemistry Instrumentation Facility (DCIF) on a Bruker Daltronics APEX II 3 Tesla FT-ICR-MS.

6.5.2 NMRP initiator/end-cap, 3

The compound 2 (300mg, 0.640mmol), Cu (61mg, 0.96mg) and CuCl (25mg, 0.26mmol) were placed in a 10mL Schlenk flask and degassed by three vacuum-Ar filling cycles. Dioxane was Ar-purged for 10 minutes. Tris[2-(dimethylamino)ethyl]amine (Me₆TREN) (59mg, 0.26mmol) in dioxane (3mL) was poured into the Schenck flask, and di-t-butyl nitroxide (125mg, 0.867mmol) was added under Ar. The reaction mixture was stirred at 70°C overnight. The reaction mixture was evaporated, and the residue was chromatographed on a silica gel column (ethyl acetate (100) to ethyl acetate (95): ethanol (5)). The collected product was dissolved in CH₂Cl₂ and washed with sat. NH₄Cl aq. solution and sat. NaCl solution. The organic layer was dried over MgSO₄, and the solvent was evaporated off after a filtration of MgSO₄ to give an oily compound (190mg, 61%). ¹H NMR (300MHz, CDCl₃): δ 7.76 (d, 2H, J = 8.1Hz), 7.53 (d, 2H, J = 8.7Hz), 6.89 (bs,
2H), 4.31 (q, 1H, J = 6.9Hz), 3.64-3.41 (m, 12H), 1.45 (d, 3H, J = 6.9Hz), 1.21 (s, 18H);

$^{13}$C NMR (75MHz, DMSO-$d_6$): 173.91, 166.77, 137.82, 134.03, 128.854, 98.65, 82.11,
70.55, 70.19, 70.05, 40.10, 38.90, 31.06, 30.61, 19.06; HR-MS (ESI) calcd. for
C$_{24}$H$_{49}$IN$_3$O$_5$: 600.1905(M$^+$+Na), found: 578.2081 (M$^+$+H), 600.1880 (M$^+$+Na),
1177.3847 (2M$^+$+Na).

6.5.3 Polymerization of PPE (macronitiator) 6

![Polymerization Diagram]

Monomers 4 (87.3mg, 0.0779mmol), 5 (40.8mg, 0.0909mmol) and alkoxyamine 2
(10mg, 0.0173mmol) were placed in 25mL Schlenk flask. Pd(PPh$_3$)$_4$ (5%) and CuI (5%)
were added to the flask in a glove box. Morpholine was passed through an alumina
column and degassed by three quick vacuum and back-filled with Ar cycles. The solvent
was cannulated into the flask containing the monomers (~0.5mL). The reaction mixture
was stirred at 60°C overnight and poured into EtOAc. The precipitate was collected by
centrifuge and washed with EtOAc, diethyl ether and dried under vacuum. The
compound was dissolved in water and dialyzed against water (Pierce, Snake skin,
MWCO 3500) for 2 days (500ml water x 4 times in a day). The polymer solution was
lyophilized (86mg). $^1$H NMR (300MHz, DMSO-$d_6$): δ 7.75 (bs, 2H), 7.451 (bs, 2H),
7.074 (bs, 4H), 4.951 (bs, 16H), 4.596 (s, 8H), 4.098 (bs, 4H), 3.988 (bs, 4H), 3.560 (bs, 40H), 1.201 (s, 1.83H), 1.144 (s, 1.83H).

6.5.4  Polymerization of PPBT 8

Monomers 4 (48.5 mg, 0.043mmol), 7 (23.4 mg, 0.00476mmol) and alkoxyamine 2 (5mg, 0.009mmol) were placed in 25mL Schlenk flask. Pd(PPh₃)₄ (5%) was added to the flask in a glove box. DMF was degassed by three quick vacuum and back-filled with Ar cycles and cannulated into the Schlenk flask (1mL). The reaction mixture was stirred at 80°C overnight and poured into EtOAc. The precipitate was collected by centrifuge and washed with ethyl acetate and diethyl ether and dried under vacuum. The compound was dissolved in water and dialyzed against water (Pierce, Snake skin, MWCO 3500). The polymer solution was lyophilized after filtration. ¹H NMR (300MHz, DMSO-d₆): δ 8-7 (bm, 10H), 4.632 (bs, 4H), 4.15 (bs, 8H), 3.64-3.30 (bm, 24H), 1.197 (s, 0.7H), 1.137 (s, 0.7H).

6.5.5  General procedure of NMRP of NIPA with PPE or PPBT

PPE (10mg) and NIPA (500mg) were placed in a 10mL Schlenk flask and degassed by three vacuum-Ar filling cycles. Ar-bubbled DMF or DMSO (0.5mL) was
added to the flask by syringe. The reaction mixture was stirred at 120°C overnight. The polymer was precipitated out in ethyl ether and collected by centrifuge. The dried polymer was dissolved in water and purified by a dialysis against water. The polymer solution was lyophilized to give a yellow powder, 22mg.

6.5.6 Synthesis of RhB-polyNIPA IV

NIPA (0.50g, 4.4mmol) and methacyloxyethyl thiocarbamoyl rhodamine B (7.4mg, 0.011mmol, Polysciences, Inc.), AIBN (3.6mg, 0.022mmol) were placed in a 25mL Schlenk tube and degassed by three vacuum-Ar filling cycles. Ar-bubbled DMF (1.5mL) was added to the tube by syringe. The reaction mixture was stirred at 60°C overnight. The polymer was precipitated out in ethyl ether and collected by centrifuge and dried under vacuum.

6.5.7 Synthesis of PPE-RhB-polyNIPA V

PPE (5mg), NIPA (0.5g) and methacyloxyethyl thiocarbamoyl rhodamine B (0.4mg, 0.6mmol, Polysciences, Inc.) were placed in a 25mL Schlenk tube and degassed
by three vacuum-Ar filling cycles. Ar-bubbled DMSO (0.5mL) was added to the tube by syringe. The reaction mixture was stirred at 120°C overnight. The polymer was precipitated out in ethyl ether and collected by centrifuge. The dried polymer was dissolved in water and purified by a dialysis against water (Pierce, Snake skin, MWCO 3500). The polymer solution was lyophilized.

6.5.8 General protocol for thermal precipitation

Polymer stock solutions (20mL) were prepared by stirring the polymers in deionized water and cooling in an ice bath. The polymer concentrations were 0.15mg/mL for PPE-polyNIPA I and 0.25mg/mL for RhB-polyNIPA IV to give about 0.5 and 0.4 at the maximum in UV-vis absorbance, respectively. The stock solutions were diluted to the desired concentration, and they were mixed to give polymer sample solutions. The sample solution (1mL) was transferred to a 25mL sample tube, and a saturated NaCl aqueous solution (0.1mL) was added into the tube. The solution was heated at 50°C in an oil bath without stirring for 3 minutes. The resultant precipitate was vacuum-filtered with a filter paper (S&S filter paper, medium, pore size 8-12μm, diameter 8mm) on a Hirsch funnel and washed with a hot water (50°C, 3mL) without a salt. The filter paper was dried under vacuum overnight and placed between a cover slip and a glass microscope slide. Fluorescence from this assembly was measured.
Compound 3:

$^1$H-NMR (300MHz, CDCl$_3$)

$^{13}$C-NMR (75MHz, CDCl$_3$)
6: $^1$H-NMR (300MHz, DMSO-$d_6$)
8: $^1$H-NMR (300MHz, DMSO-$d_6$)
6.6 References


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Curriculum Vitae

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Thesis title: Thermally Responsive Polymers and Their Applications
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1995-1997  Kyoto University  Kyoto, Japan
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Professional Experience
1997-2003  Graduate Research Assistant  Massachusetts Institute of Technology
Advisors: Profs. Timothy Swager and Toyoichi Tanaka
- Investigated new water-soluble conjugated polymers for sensing biomolecules
- Prepared conjugated polymers modified with temperature-sensitive polymers and studied the solution properties
- Developed catalysts using polymer gel
- Studied volume phase transitions in polymer gels

1994-1997  Graduate Research  Kyoto University, Japan
Advisors: Profs. Junzo Sunamoto and Kazunari Akiyoshi
- Synthesized hydrophobically modified polysaccharide and characterized them by spectroscopic methods and light scattering techniques
- Investigated gel formation of the polymers in water by rheological methods

Publications