A NEW CLASS OF BIODEGRADABLE, COACERVATE-FORMING, 
THERMORESPONSIVE POLYESTERS BASED 
ON N-SUBSTITUTED DIOLS

A Dissertation
Presented to
The Graduate Faculty of The University of Akron

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

John P. Swanson
May, 2016
A NEW CLASS OF BIODEGRADABLE, COACERVATE-FORMING, THERMORESPONSIVE POLYESTERS BASED ON N-SUBSTITUTED DIOLS

John P. Swanson
Dissertation

Approved:

Advisor
Dr. Abraham Joy

Committee Member
Dr. Matthew L. Becker

Committee Member
Dr. Mesfin Tsige

Committee Member
Dr. Ali Dhinojwala

Committee Member
Dr. Nic D. Leipzig

Accepted:

Department Chair
Dr. Coleen R. Pugh

Dean of the College
Dr. Eric J. Amis

Dean of the Graduate School
Dr. Chand Midha

Date

ii
ABSTRACT

Temperature-responsive smart materials, such as poly(N-isopropylacrylamide) (PNIPAM), undergo a reversible hydrophilicity change at a lower critical solution temperature (LCST) which makes them particularly attractive for biomedical applications such as targeted drug delivery. However, the nondegradable backbone of PAMs and many other thermoresponsive polymers ultimately limit their use for a number of applications. To date, few examples of thermoresponsive, biodegradable polymers exist. In looking to overcome these limitations, our lab has developed a library of thermoresponsive polyesters (TR-PEs) based on PAs and thermoresponsive elastin-like peptides (ELPs). A modular synthetic design allows for polyesterification of a variety of N-substituted diol monomers, yielding a library of TR-PEs. The LCST range can be tuned between 0-100 °C, dependent on polymer structure and cosolutes, as evidenced by UV-vis, 1H NMR, and DLS. Additionally, the hydrophilic nature of TR-PEs prevents full dehydration above the LCST, resulting in the formation of polymer-rich coacervates capable of encapsulating hydrophobic small molecules and proteins. The degradable polyester backbone enables hydrolytic degradation over time. These features make TR-PEs attractive candidates for applications such as controlled drug release and degradable scaffolds.
DEDICATION

I would like to dedicate this dissertation to my mother, for always encouraging my scientific curiosity, and to Phil Costanzo, for giving that curiosity direction.
ACKNOWLEDGEMENTS

I am incredibly fortunate to have received support from so many during my academic career. I would like to thank my advisor, Dr. Abraham Joy, for his guidance, support, patience, and willingness to listen to my many eccentric ideas. I’d furthermore like to thank the members of my Ph.D. committee, Dr. Matthew Becker, Dr. Ali Dhinnojwala, Dr. Mesfin Tsige, and Dr. Nic Leipzeig for their valuable feedback and thoughtful comments. I am extremely thankful for the members of the Joy Laboratory, especially Dr. Ying Xu and Dr. Sachin Gokhale for pioneering the work upon which this publication is based, Steven Mankoci for his industrious biology work, my in-lab collaborators Leanna Monteleone, Michael Martinez, and Megan Cruz, for consistently knockin’ out exciting results, and Dr. Fadi Haso from Dr. Tianbo Liu’s lab for teaching me to love scattering. I am exceptionally grateful to have received invaluable mentorship during my undergrad years. My great thanks go to Dr. Rod Schoonover and the brothers of the Gamma Zeta Chapter of Alpha Chi Sigma for opening the doors to my scientific career and teaching me the value of community in the sciences. I am likewise deeply grateful to my collaborator and undergraduate advisor Dr. Philip Costanzo, whose advice and encouragement inspired me to pursue graduate studies.

This publication would not have been possible without the overwhelming support of my family and friends. My mother’s encouragement and faith in me gave me the confidence no amount of failed experiments could deter. My brother’s esteem and respect has
pushed me to live up to the person he sees. Many amazing people have helped make Akron a home to me over the years. I would like to express my gratitude to Dr. Bill Storms-Miller, Gina Policastro, and Stefanie Wise. Without their advice, inspiration, and encouragement over pints & wine, I would have likely evaporated.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF SCHEMES</td>
<td>xv</td>
</tr>
</tbody>
</table>

## CHAPTER

I. INTRODUCTION ........................................................................................................... 1

II. LITERATURE REVIEW ................................................................................................ 4

   2.1 Introduction ...................................................................................................... 4
   2.2 Mechanism of Action .......................................................................................... 7
   2.3 Thermoresponsive Polymer Systems and Biomedical Applications .................. 13
   2.4 Synthetic, Biodegradable Polymers .................................................................. 24
   2.5 Examples of synthetic biodegradable LCST-type polymers .............................. 29
   2.6 Carbodiimide-mediated Polyesterification ..................................................... 35

III. EXPERIMENTAL METHODS ......................................................................................... 39

   3.1 Materials .......................................................................................................... 39
   3.2 Techniques ......................................................................................................... 40

IV. N-SUBSTITUTED DIOL MONOMER SYNTHESIS ............................................................. 45

   4.1 Introduction ...................................................................................................... 45
   4.2 Synthesis of N,N-bis(hydroxyethyl) Alkyl & Alkoxy Monomers (HEA monomers) ................................................................. 45
   4.3 Synthesis of Succinamide Esters ......................................................................... 50
4.4 Synthesis of \( N,N\)-bis(hydroxyethyl) Succinamide Monomers (HESA monomers) .................................................................................................................. 58

V. POLYESTERIFICATION OF N-SUBSTITUTED DIOL MONOMERS ............. 68

5.1 Introduction............................................................................................................. 68

5.2 General Procedure for Polyesterification of \( N\)-Substituted Diol Monomers ................................................................................................................................. 68

5.3 General Procedure for Polyesterification of \( N\)-Substituted Amide Diol Monomers ................................................................................................................................. 72

VI. FIRST GENERATION THERMORESPONSIVE POLYESTERS ......................... 84

6.1 Introduction............................................................................................................. 84

6.2 Synthesis and Characterization of Monomers and Polyesters ................. 86

6.3 Thermally Induced Phase Transitions................................................................. 88

6.4 Effect of Polyester Concentration, Molecular Weight, and Cosolutes on Phase Transition ........................................................................................................ 92

6.5 Tuning Phase Transition Temperature via Copolymerization ................. 94

6.6 Coacervate Analysis ............................................................................................ 96

6.7 Nile Red Separation ............................................................................................ 98

6.8 Degradation Behavior ....................................................................................... 99

6.9 Conclusion ........................................................................................................... 100

6.10 Copyright notice ............................................................................................... 101

VII. SECOND GENERATION THERMORESPONSIVE POLYESTERS .............. 102

7.1 Introduction............................................................................................................. 102

7.2 Synthesis and Characterization of Monomers and Polyesters ................. 103

7.3 Thermoresponsive Behavior .............................................................................. 105

7.4 Effects of Concentration ................................................................................... 107

7.5 Effect of Molecular Weight ............................................................................... 108

7.6 Thermal Cycle Testing ....................................................................................... 108
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>LCST of select polymers.</td>
<td>15</td>
</tr>
<tr>
<td>2.2</td>
<td>pH in various biological tissues.</td>
<td>29</td>
</tr>
<tr>
<td>6.1</td>
<td>Characterization of TR-PEs</td>
<td>92</td>
</tr>
<tr>
<td>6.2</td>
<td>Characterization of TR-PE Copolyesters</td>
<td>94</td>
</tr>
<tr>
<td>7.1</td>
<td>Characterization of TR-(bMoEtA-r-iPrA)PE Copolyesters</td>
<td>112</td>
</tr>
<tr>
<td>8.1</td>
<td>Characterization of amide TR-PEs.</td>
<td>126</td>
</tr>
<tr>
<td>8.2</td>
<td>Solution properties of amide TR-PEs.</td>
<td>128</td>
</tr>
<tr>
<td>8.3</td>
<td>Characterization of alkyl &amp; alkoxy TR-PEs.</td>
<td>133</td>
</tr>
<tr>
<td>8.4</td>
<td>Solution properties of alkyl &amp; alkoxy TR-PEs.</td>
<td>133</td>
</tr>
<tr>
<td>9.1</td>
<td>Characterization of the solution properties of ionic Glu-TR-PEs</td>
<td>144</td>
</tr>
<tr>
<td>9.2</td>
<td>Characterization of the solution properties of ionic HEA-TR-PEs</td>
<td>149</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Temperature vs. polymer volume fraction, $\phi$. Schematic illustration of phase diagrams for polymer solution (a) lower critical solution temperature (LCST) behavior and (b) upper critical solution temperature (UCST) behavior. Reprinted with permission from Science, 2009. 12</td>
</tr>
<tr>
<td>2.2</td>
<td>Published items and publications per year by Web of Knowledge, searching the term “thermoresponsive polymer”, March 2016.</td>
</tr>
<tr>
<td>2.3</td>
<td>Thermoresponsive copolymer architectures. The copolymer conformations are depicted at below the LCST. The red segments are thermoresponsive while the blue segments are non-thermoresponsive. Reprinted with permission from Springer, 2009.</td>
</tr>
<tr>
<td>2.4</td>
<td>Interactions among anions, PNIPAM, and hydration waters. (a) Hydrogen bonding of the amide and its destabilization through polarization by the anion, X$^{-}$. (b) The hydrophobic hydration of the molecule is associated with surface tension and can be modulated by salt. (c) Direct binding of the anion to the amide group of PNIPAM. Reprinted with permission from The American Chemical Society, 2005.</td>
</tr>
<tr>
<td>2.5</td>
<td>(A) Concentration effect on the LCST of aqueous PDEAM measured by DSC (squares) at a heating rate of 5 °C min$^{-1}$ and by UV–vis (circles) at 0.25 °C min$^{-1}$ ($\lambda = 500$ nm). (B) Effect of heating rate on the LCST of a 2 wt % PDEAM solution measured by DSC (squares) and UV–vis spectrophotometry ($\lambda = 500$ nm). Reprinted with permission from The American Chemical Society, 1999.</td>
</tr>
<tr>
<td>2.6</td>
<td>Select thermoresponsive polymers bearing both hydrophobic and hydrophilic moieties.</td>
</tr>
<tr>
<td>2.7</td>
<td>Temperature-modulated drug release and interactions between micelles with PNIPAM as shell-forming segments and cells. Reprinted with permission from Elsevier, 1999.</td>
</tr>
<tr>
<td>2.8</td>
<td>The homologue series of PAOxs that share the polar amide motif with decreasing water solubility as the 2-alkyl side chain length increases. PAOxs with intermediate side chain length display a temperature dependent solubility. Reprinted with permission from Elsevier, 2010.</td>
</tr>
</tbody>
</table>
2.9 Synthesis of PPCN copolymer and formation of branched, antioxidant PPCN gels.\textsuperscript{79} Reprinted with permission from The American Chemical Society, 2014. 

2.10 Hydrolytically degradable linkages used in biomaterials.\textsuperscript{132, 134} ................................ 28

2.11 Select degradable thermoresponsive homo- and copolymers. Hydrolytically cleavable bonds are shown in red, LCST or $T_{cp}$ (°C) are shown in parentheses.\textsuperscript{31}

6.1 Chemical structure of poly(acrylamide)s (PAMs), poly(alkyloxazoline)s (PAOxs), elastin-like poly(peptide)s (ELPs), “peptide-like” poly(ester)s, and thermoresponsive poly(ester)s (TR-PE, this work). ........................................ 85

6.2 Thermoresponsive polymers as the inspiration for TR-PE library. ....................... 85

6.3 TR-PE library. .................................................................................................................. 90

6.4 Reversible cloud point behavior of TR-PyrAPE (top, $T_{cp} = 15.8$ °C, 10 mg/mL) and temperature-dependent transmittance of TR-iPrAPE, TR-dEtAPE, and TR-PyrAPE (bottom, $M_n \sim 55$ kDa, 10 mg/mL, 1 °C/min) in DI water exhibiting clear hysteresis. ........................................................................................................ 90

6.5 Variable temperature \textsuperscript{1}H NMR spectra of TR-PyrAPE ($T_{cp} = 15.8$ °C) in D$_2$O above and below cloud point. ...................................................................................... 91

6.6 Aqueous solution properties of TR-PEs (10 mg/mL unless otherwise stated) as a function of concentration (A), molecular weight (B), NaCl (C), and SDS (D), and urea (E). .................................................................................................................. 93

6.7 Temperature-dependent transmittance of TR-PE copolyester solutions ($M_n \sim 55$ kDa). Trend line is added as a visual guide. ......................................................................................... 96

6.8 CONTIN analysis of the DLS data of TR-dEtAPE ($T_{cp} = 11.8$ °C) above and below the cloud point .................................................................................................................... 98

6.9 Optical micrograph (0.5 wt %, 40× magnification) of TR-dEtAPE ($M_n = 55$ kDa, $T_{cp} = 11.9$ °C) showing coacervate droplets at room-temperature under bright-field filter (A) and after introduction of Nile Red under bright-field (B) and TRITC filters (C). Scale bar is the same for all images. .................................................... 99

6.10 Hydrolytic degradation of TR-dEtAPE over a period of 7 days; n = 3. .......... 100

7.1 \textsuperscript{1}H NMR spectra of TR-bMoEtAPE (300 MHz, CDCl$_3$) ........................................ 105

7.2 $T_{cp}$ investigations of TR-bMoEtAPE (132 kDa) using UV–vis (500 nm, 1 °C/min heat/cool, 1 wt % PE solution unless otherwise noted) (A) UV–vis spectra of TR-bMoEtAPE (132 kDa) showing hysteresis of initial heating and cooling cycles. (B) The effect of TR-bMoEtAPE concentration on $T_{cp}$. (C) The effect of
molecular weight on \( T_{cp} \). (D) Thermal reversibility of TR-bMoEtAPE over six cycles. (E) The effect of urea and various Hofmeister anions on \( T_{cp} \).

7.3 Effect of copolyester composition on \( T_{cp} \) and \( T_g \) for a series of TR-(bMoEtA-r-iPrA)PE of various \( M_n \). Dashed lines represents theoretical values using the Fox equation (blue) and a weighted average (black).

7.4 (A) Variable temperature \(^1\text{H} \) NMR spectra of TR-bMoEtAPE (400 MHz, D\(_2\)O, \( T_{cp} = 48.1 ^\circ \text{C} \)) above and below cloud point and (B) the normalized proton integral signals.

7.5 CONTIN analysis of the DLS data of TR-bMoEtAPE (\( T_{cp} = 53.5 ^\circ \text{C} \)) above and below the cloud point.

7.6 Left: Hydrolytic degradation via SEC of TR-bMoEtAPE incubated 37 °C in DI water; n = 3. Right: hydrolytic degradation via UV–vis of TR-bMoEtAPE incubated at 37 °C in 1X PBS buffer.

7.7 Cell viability of TR-bMoEtAPE against NIH 3T3 cells, 1 day; n = 3.

8.1 Library of investigated TR-PE with varying residue structure.

8.2 Temperature-dependent transmittance of TR-PEs DI water (right) and 1X PBS buffer (left) exhibiting clear hysteresis between heating (solid lines) and cooling (dashed lines).

8.3 The effect of molecular weight on \( T_{cp} \) for TR-PEs. (10 mg/mL, 1 °C/min heating rate, DI water). Trend lines are added as a visual aid. TR-MoEtAPE is soluble below \( M_n \sim 35 \text{ kDa} \).

8.4: The \( T_{cp} \) of amide TR-PEs as a function of \( T_g \) (A), carbon and oxygen number of amide substituents (B), and O/C ratio of amide substituents (C).

8.5 \(^1\text{H} \) NMR spectra and UV–vis traces (inset) of TR-(90MoMe-r-10ibuDEA)PE (top) and TR-(90MeEtA-r-10ibuDEA)PE (bottom) (500 MHz, CDCl\(_3\)).

9.1 Temperature driven encapsulation and release of protein by ionic TR-PEs.

9.2 \(^1\text{H} \) NMR spectra of cPrA4 before (bottom, black) and after (top, red) deprotection showing complete removal of the Boc group. (500 MHz, DMSO-d\(_6\)).

9.3 Temperature-dependent phase behavior of iPrA3 and cPrA3 (5 mg/mL) at pH 6-8 in the presence and absence of BSA (0.5 mg/mL) (500 nm, 1 °C heating).

9.4 Optical micrographs (20x magnification, FITC filter) of cPrA4 and cPrA5 coacervates (5 mg/mL) in 100 mM PB containing FITC-BSA (0.5 mg/mL) at varying pH. Scale bar is 50 um.
10.1 Overview of TR-PE library................................................................. 151
10.2 TR-PE library based on amide (Z) or alkyl and alkoxy (Y) residues......... 152
10.3 Ionic TR-PEs for thermoresponsive protein encapsulation...................... 153
<table>
<thead>
<tr>
<th>Scheme</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Polymerization of 2-oxazolines as well as the structural analogy with poly(amino acid)s and PAMs. (^{37})</td>
</tr>
<tr>
<td>2.2</td>
<td>Polymerization and subsequent deprotection of functionalized (\varepsilon)-caprolactone monomers. (^{181})</td>
</tr>
<tr>
<td>2.3</td>
<td>The Steglich esterification using DCC and DMAP.</td>
</tr>
<tr>
<td>2.4</td>
<td>Polyesterification of a semi-aromatic hydroxy acid via DIC/DPTS coupling.</td>
</tr>
<tr>
<td>4.1</td>
<td>Synthetic route for preparation of HEA monomers.</td>
</tr>
<tr>
<td>4.2</td>
<td>Synthetic route for preparation of succinamide ester intermediates.</td>
</tr>
<tr>
<td>4.3</td>
<td>Synthetic route for preparation of HESA monomers.</td>
</tr>
<tr>
<td>6.1</td>
<td>Synthetic Route for the Preparation of PEs. Reagents and conditions: (i) DEA, neat, 80 °C, 16 h; (ii) SA, DIC, DPTS, CH(_2)Cl(_2), 0 °C to room-temperature, 48 h; (iii) ethylsuccinyl chloride, Et(_3)N, CH(_2)Cl(_2), 0 °C to room-temperature, 1 h.</td>
</tr>
<tr>
<td>7.1</td>
<td>Synthetic route for the preparation of TR-bMoEtAPE. Reagents and conditions: (i) Et(_3)N, CH(_2)Cl(_2), 0 °C to room-temperature, 1 h. (ii) DEA, neat, 80 °C, vacuum, 16 h. (iii) SA, DIC, DPTS, CH(_2)Cl(_2), 0 °C to room-temperature, 48 h.</td>
</tr>
<tr>
<td>8.1</td>
<td>Synthetic route for the preparation of TR-bMoEtAPE. Reagents and conditions: (i) Et(_3)N, CH(_2)Cl(_2), 0 °C to room-temperature, 1 h. (ii) DEA, neat, 80 °C, vacuum, 16 h. (iii) Et(_3)N, MeOH, 60 °C microwave, 2 h. (iv) Ibuprofen, DIC, DPTS, CH(_2)Cl(_2), 0 °C to room-temperature, 16 h. (v) DEA, neat, 70 – 80 °C microwave, 30 min. (vi) SA, DIC, DPTS, CH(_2)Cl(_2), 0 °C to room-temperature, 48 h.</td>
</tr>
<tr>
<td>9.1</td>
<td>Synthetic route for preparation of ionic TR-PEs.</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

Thermoresponsive polymers exhibiting an aqueous Lower Critical Solution Temperature (LCST), such as poly(N-isopropylacrylamide) (PNIPAM), have been widely investigated in the biomaterials field for possible applications within tissue engineering, purification, and as smart drug delivery vehicles.\textsuperscript{1-4} Of note are thermoresponsive polymers which do not fully dehydrate but instead form polymer-rich coacervates, making them particularly attractive for the delivery of sensitive biomolecules.\textsuperscript{5-8} However, the non-degradability of many aqueous LCST polymers can limit applications. To date, few examples of fully degradable thermoresponsive polymers exist. This can be partially attributed to the difficulty of designing thermoresponsive polymers as a number of factors influence the LCST (if one is observed at all).

This work builds upon prior research by Gokhale and Xu in our lab, who used room-temperature carbodiimide-mediated polymerization to synthesize a library of biodegradable “peptide-like” homo- and random polyesters (PEs) with a variety of bio-inspired pendant groups.\textsuperscript{9} Similar to polypeptides, these PEs contain a significant amount of oxygen and nitrogen atoms in the polymer backbone as they are based on \textit{N}-substituted diols. Despite the relatively hydrophilic backbone, the “peptide-like” PEs did not display room-temperature aqueous solubility or thermoresponsive behavior. As the “peptide-like” PEs
displayed similar structures to a variety of thermoresponsive polymers such as poly(acrylamide)s (PAMs), poly(alkyloxazoline)s (PAOs), and elastin-like peptides (ELPs), it was hypothesized that a pendant group could be designed that would shift the overall hydrophobic/hydrophilic balance of the polymer and result in LCST behavior.

This publication focuses on the development of biodegradable, thermoresponsive polyesters (TR-PEs). A variety of N-substituted monomers bearing amide, alkyl, and alkoxy pendant groups were synthesized by a neat, one-pot transamidation reaction with diethanolamine. Room-temperature carbodiimide-mediated polyesterification allowed for a simple route to high molecular weight TR-PEs with relatively low polydispersity ($D_M < 1.5$). TR-PEs were characterized using SEC, TGA, DSC, and NMR.

Upon heating, a number of aqueous TR-PE solutions displayed a clear optical change from clear to cloudy. This macroscopic change, known as the cloud point temperature ($T_{cp}$), corresponds to LCST behavior. However, traditional routes to accurately determine LCST, such as DSC, were initially unsuccessful. The significant hydrophilic components of the TR-PEs prevented complete dehydration above the LCST, resulting instead in an unexpected coacervation-type response that was proved via UV-Vis, DLS, $^1H$ NMR, and water content.

The first generation TR-PEs displayed low LCST values which led to the development of second generation TR-PEs based on a more hydrophilic monomer. The second generation TR-PEs allowed for greater tuning of thermal, physical, and solution properties via copolymerization as evidenced by DSC and UV-Vis. The design of future smart-materials based on the TR-PE system requires insight into the tunability of polymer physical,
thermal, and solution properties. To this end, the TR-PE homopolymer library was expanded to 20 entries. Preliminary investigations suggest that TR-PEs are non-cytotoxic, can maintain thermoresponsivity when covalently attached to hydrophobic model drugs, and are capable of thermoresponsive encapsulation of model compounds and proteins.
CHAPTER II

LITERATURE REVIEW

2.1 Introduction

Stimuli-responsive, or “smart”, materials have found wide acceptance across a variety of fields due to their ability to exhibit a significant change in physical properties as a result of a minor change in external stimuli such as light, electric potential, pH, redox, magnetic field, pressure, or temperature.\textsuperscript{10-11} While it is true that all polymers in solution conformationally respond to changes in temperature, “smart” thermoresponsive polymers undergo drastic conformational changes with relatively minor changes in temperature, typically leading to a phase separation. These polymers can be broadly categorized according to their solution behavior at elevated temperatures: polymers that become soluble display Upper Critical Solution Temperature (UCST) behavior while those which become insoluble display Lower Critical Solution Temperature (LCST) behavior (Figure 2.1). The UCST and LCST, if either exist, are specific to both the polymer and the solvent.
Polymers displaying a LCST have been widely studied since their initial reports by Freeman and Rowlinson in 1960. However, over the past 20 years research into thermoresponsive polymers has exploded with a primary focus on materials which display aqueous LCST behavior (Figure 2.2). This is largely due to the appeal of LCST-type polymers within the biomedical field for applications including controlled drug and gene delivery, filtration, smart coatings, tissue engineering, filtration, and enzyme regulation.

A universal feature of all aqueous thermoresponsive homopolymers is the presence of both hydrophobic and hydrophilic domains. For example, poly(N-isopropylacrylamide) (PNIPAM, L1) contains hydrophilic amide groups as well as hydrophobic i-propyl groups and carbon backbone. Additionally, copolymerization allows for tuning of the LCST (random copolymerization) or the formation of advanced structures such as micelles/polymeromes (block copolymers) and gels (graft/comb copolymers) (Figure 2.3).
Figure 2.2: Published items and publications per year by Web of Knowledge, searching the term “thermoresponsive polymer”, March 2016.

Figure 2.3: Thermoresponsive copolymer architectures. The copolymer conformations are depicted at below the LCST. The red segments are thermoresponsive while the blue segments are non-thermoresponsive. Reprinted with permission from Springer, 2009.
2.2 Mechanism of Action

From a thermodynamic standpoint, a polymer will dissolve in a solvent if the Gibbs energy of mixing is negative ($\Delta G_{mix} < 0$) as described by the Gibbs free energy equation as applied to mixing at constant temperature and pressure:

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix}$$  \hspace{1cm} (1)

In the absence of polymer-polymer, polymer-solvent, and solvent-solvent interactions, the enthalpy of mixing ($\Delta H_{mix}$) can be ignored. The increase in entropy of mixing (negative value for $-T\Delta S_{mix}$ term) will result in $\Delta G_{mix} < 0$ and complete miscibility for this ideal system. For UCST polymer systems, the observed phase separation is primarily due to beneficial enthalpic contributions upon demixing. Conversely, LCST behavior is the result of beneficial entropy. That is, above the LCST it is more entropically favorable to have a phase separated system. This is less intuitively understood than UCST behavior.

Early statistical models to predict thermoresponsive behavior relied on equations of state such as the Flory-Huggins lattice model:\(^{16-17}\)

$$\Delta G_{mix} = RT[n_1 \ln \phi_1 + n_2 \ln \phi_2 + n_1 \phi_2 \chi_{12}]$$  \hspace{1cm} (2)

where $R$ is the gas constant, $T$ is temperature, $n_1$ and $n_2$ are the moles of solvent (component 1) and polymer (component 2), $\phi_1$ and $\phi_2$ are the volume fractions of solvent and polymer, and $\chi_{12}$ is the entropic interaction parameter between solvent and polymer. While the Flory-Huggins model is able to predict UCST behavior, it was not until the more complex Sanchez-Lacombe model,\(^{18}\) which accounted for vacancies, variable densities, and compressibility, that LCST behavior of numerous polymer systems could be predicted. However, LCST prediction by the Sanchez-Lacombe model only holds for weakly inter-
acting polymers and does not take into account hydrogen-bonding interactions that are primarily responsible for the LCST behavior of polymers in aqueous solutions. Sanchez and Panayiotou later updated this model to include hydrogen-bonding, while Simmons further expanded on Sanchez’s work to include aqueous salt solutions.

For polymers that show LCST-type response, it is worth noting what is being described by the terms “hydrophilic” and “hydrophobic” and how they impact LCST behavior. Initially, polymer dissolution is driven by hydrogen bond formation between water and “water-loving,” or hydrophilic, groups. The formation of these bonds is enthalpically favorable ($\Delta H < 0$) and lowers the overall Gibbs free energy ($\Delta G < 0$). The “hydrophobic” groups on the polymer are not truly “water-fearing” as implied by their name. There actually exists a small amount of attraction between hydrophobic groups and water molecules due to dispersion forces. However, these dispersion forces are much weaker than the attractive hydrogen bonds between water molecules. By interacting with one another instead of with water, hydrophobic moieties permit greater numbers of water molecules to hydrogen bond with one another resulting in a decrease of the overall Gibbs free energy ($\Delta G < 0$). When LCST-type polymers are dissolved at low temperatures, water molecules must adopt an entropically unfavorable ($\Delta S > 0$) ordered (or “ice-like”) shell of hydration around the hydrophobic domains. The restructuring of water molecules is allowed since the beneficial enthalpic contribution of the hydrogen bonds between water and the polar groups lowers the overall Gibbs free energy of mixing ($\Delta G_{\text{mix}} < 0$). At elevated temperatures, hydrogen-bonded and structured water molecules are released as bulk water, resulting in a massive overall entropy gain to the system ($\Delta S < 0$). As water molecules bound to the hydrophilic domains are released, their enthalpic contributions to solvation are no
longer able to counteract the local hydrophobic effects and the overall Gibbs free energy of mixing becomes positive ($\Delta G_{\text{mix}} > 0$). The polymer chain begins to self-associate (sometimes forming intramolecular hydrogen bonds), aggregate, and eventually phase separate due to the overall gain in system entropy.

Predicting whether or not a polymer will be thermoresponsive relies on a number of factors. As previously mentioned the polymer must contain both hydrophobic (typically aliphatic) groups and hydrophilic groups such as amines, amides, ethers, esters, hydroxyls, and carboxylic acids. Prediction becomes more complex in the case of copolymers. For random copolymers, the LCST is typically an average of the mean residue hydrophobicity and is influenced by overall composition. For polymers containing blocks, each segment collapses at the homopolymer LCST which can be used to form and break micelles or cause gelation. Second, molecular weight must be taken into account especially as low molecular weight polymers are typically more influenced by the hydrophobicity of chain ends. Hydration also generally decreases with increasing molecular weight as a result of increased polymer-polymer interactions, thus lowering the phase transition temperature.

Solution conditions such as osmolarity, additives, and pH can influence the LCST. Therefore, when considering thermoresponsive polymers for use as biomaterials, local in-vivo osmotic concentration and pH must be taken into account. As explained by the model put forward by Cremer and coworkers, for the case of large divalent or small monovalent anions such as Cl$^-$, the high charge density results in the anion becoming strongly solvated in solution. The hydrated anion is able to interact with the hydration shell of the polymer, promoting dehydration of the hydrophobic and hydrophilic segments which re-
sults in reduced polymer solubility and LCST (“salting-out” effect, Figure 2.4A). Conversely, large monovalent anions such as SCN⁻ contain less charge density and are more polarizable, resulting in the anions being less hydrated in solution. The large monovalent anions are able to bind directly to hydrophobic groups via dispersion forces and to hydrophilic groups via ion-dipole interactions which increases polymer solubility and LCST (“salting-in” effect, Figure 2.4C). The ability for the anions of cosalts to salt-out or salt-in a thermoresponsive polymer generally follows the Hofmeister’s series: CO₃²⁻ > SO₄²⁻ > S₂O₃²⁻ > H₂PO₄²⁻ > F⁻ > Cl⁻ > Br⁻ > I⁻ > ClO₄⁻ > SCN⁻.

Figure 2.4: Interactions among anions, PNIPAM, and hydration waters. (a) Hydrogen bonding of the amide and its destabilization through polarization by the anion, X⁻. (b) The hydrophobic hydration of the molecule is associated with surface tension and can be modulated by salt. (c) Direct binding of the anion to the amide group of PNIPAM. Reprinted with permission from The American Chemical Society, 2005.

Ionic surfactants, such as sodium dodecyl sulfate (SDS), interact with the polymer both below and primarily above the LCST. Below the LCST, surfactant molecules are able to associate with hydrophobic groups and form micelles along the polymer backbone. This serves to stabilize the polymer and ultimately increase the LCST. Additionally, above the LCST surfactant micelles solubilize collapsed hydrophobic polymer globules, preventing their aggregation and increasing the onset of the observed cloud point temperature (discussed more in depth below). For polymers containing ionizable groups such as poly(2-dimethylaminoethyl methacrylate) (PDMAEMA, L23), pH is an important factor. Adding charge to the polymer increases its overall hydrophilicity and increases the LCST.
Since the LCST is a thermodynamic quantity, its determined value should not depend on the experimental method. Differential scanning calorimetry (DSC) has proven to be one of the most accurate and robust methods of exploring the LCST for aqueous thermoresponsive polymers. On a molecular level, the LCST describes the temperature at which a polymer changes from a hydrated random coil to a hydrophobic globule. The first step of this transition involves the breaking of structured (“ice-like”) waters around the solvated polymer. Upon heating hermetically sealed solutions in DSC, an endothermic peak will be observed at the temperature which the structured waters are released as bulk water. This temperature (recorded as either the endotherm onset or maxima) is typically reported as the LCST value as it correlates to the beginning of the multi-step dehydration process at the molecular level. From these data, the enthalpy of phase separation can also be calculated allowing for a qualitative measure of the hydrogen bonding strength between the polymer and bound water. However, for polymers in which the dehydration is less efficient and the enthalpic penalty does not make up for the entropic gain of ordered-to-bulk water, no peak associated with LCST is observed. This is the case for many coacervate-forming thermoresponsive polymers (to be discussed in further detail below) as well as polymers containing extremely hydrophilic comonomers.

For difficult systems, more precise methods such as Temperature Modulated DSC (TMDSC) can be used to accurately determine the LCST,25-26 but often quicker and less accurate methods are used to approximate the value. Changes in viscosity and gel points can be monitored by rheometry, particle size and aggregation can be probed using light scattering, but the most popular qualitative method is turbidity as determined by UV–vis
spectroscopy because of its simplicity and widespread availability. Above the LCST, dehydrated polymer globules begin to aggregate due to hydrophobic interactions. Once the aggregates reach a certain size, the solution will optically change from clear to cloudy. The temperature at which this observable macroscopic transformation occurs is defined as the cloud point temperature ($T_{cp}$) and is often used as a qualitative measure of the LCST. However, there is a lack of consensus as to which point constitutes $T_{cp}$ resulting in a number of reported definitions: the temperature at the onset of cloudiness, the temperature at a specific value of transmittance (such as $T = 10\%$ or $50\%$), the temperature at the inflection point of transmittance, or the temperature of the intersection of the baseline with the tangent to the inflection in transmittance.\cite{27} For certain polymers which undergo sharp cloud point transitions, such as PNIPAM, the approximation of $T_{cp}$ with LCST is fairly accurate but typically higher than the true LCST. This is because the $T_{cp}$ is affected by the kinetics of dehydration and aggregation, not only the thermodynamic quantities measured with DSC. Since the aggregation of dehydrated polymer particles takes time and UV–vis will not detect aggregates below the selected wavelength (typically 300-500 nm), $T_{cp}$ results are heavily influenced by experimental conditions such as heating rate, solution concentration, and wavelength selection. This makes UV–vis a less robust and accurate method for LCST determination than DSC as is shown for aqueous solutions of poly$(N,N$-diethylacrylamide) (PDEAM) in Figure 2.5.
Figure 2.5: (A) Concentration effect on the LCST of aqueous PDEAM measured by DSC (squares) at a heating rate of 5 °C min\(^{-1}\) and by UV–vis (circles) at 0.25 °C min\(^{-1}\) (\(\lambda = 500\) nm). (B) Effect of heating rate on the LCST of a 2 wt % PDEAM solution measured by DSC (squares) and UV–vis spectrophotometry (\(\lambda = 500\) nm).\(^{28}\) Reprinted with permission from The American Chemical Society, 1999.

2.3 Thermoresponsive Polymer Systems and Biomedical Applications

In order for thermoresponsive polymers to be useful for biomedical applications, they must exhibit minimal toxicity and a predetermined thermal response for the desired application. Typically, this ranges between room to body temperature (20 – 37 °C). While numerous thermoresponsive polymer systems have been studied as potential biomaterials for which many excellent reviews exist,\(^{4, 14, 29-41}\) a few of the most popular and relevant to this dissertation will be discussed below.
Figure 2.6: Select thermoresponsive polymers bearing both hydrophobic and hydrophilic moieties.
Table 2.1: LCST of select polymers.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>LCST or $T_{cp}$ (°C)</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNIPAM</td>
<td>32&lt;sup&gt;42&lt;/sup&gt;</td>
<td>L1</td>
</tr>
<tr>
<td>PDEAM</td>
<td>33&lt;sup&gt;28&lt;/sup&gt;</td>
<td>L2</td>
</tr>
<tr>
<td>PNNPAM</td>
<td>23&lt;sup&gt;33&lt;/sup&gt;</td>
<td>L3</td>
</tr>
<tr>
<td>PNCPAM</td>
<td>53&lt;sup&gt;44&lt;/sup&gt;</td>
<td>L4</td>
</tr>
<tr>
<td>PAPR</td>
<td>51&lt;sup&gt;45&lt;/sup&gt;</td>
<td>L5</td>
</tr>
<tr>
<td>PbMoEA</td>
<td>54&lt;sup&gt;46&lt;/sup&gt;</td>
<td>L6</td>
</tr>
<tr>
<td>PEOeEA</td>
<td>38&lt;sup&gt;47&lt;/sup&gt;</td>
<td>L7</td>
</tr>
<tr>
<td>PMoEA</td>
<td>Sol.&lt;sup&gt;48&lt;/sup&gt;</td>
<td>L8</td>
</tr>
<tr>
<td>PTHFA</td>
<td>43&lt;sup&gt;49&lt;/sup&gt;</td>
<td>L9</td>
</tr>
<tr>
<td>PAmorA</td>
<td>Sol.&lt;sup&gt;48&lt;/sup&gt;</td>
<td>L10</td>
</tr>
<tr>
<td>PMeOx</td>
<td>Sol.&lt;sup&gt;50&lt;/sup&gt;</td>
<td>L11</td>
</tr>
<tr>
<td>PEOx</td>
<td>62-65&lt;sup&gt;51&lt;/sup&gt;</td>
<td>L12</td>
</tr>
<tr>
<td>PiPrOx</td>
<td>36&lt;sup&gt;52&lt;/sup&gt;</td>
<td>L13</td>
</tr>
<tr>
<td>PcPrOx</td>
<td>30&lt;sup&gt;53&lt;/sup&gt;</td>
<td>L14</td>
</tr>
<tr>
<td>PnPrOx</td>
<td>24&lt;sup&gt;54&lt;/sup&gt;</td>
<td>L15</td>
</tr>
<tr>
<td>PnBuOx</td>
<td>&lt; 0&lt;sup&gt;50&lt;/sup&gt;</td>
<td>L16</td>
</tr>
<tr>
<td>ELPs</td>
<td>27 (VPGVG)&lt;sup&gt;55&lt;/sup&gt;</td>
<td>L17</td>
</tr>
<tr>
<td>PPO</td>
<td>0-50&lt;sup&gt;56&lt;/sup&gt;</td>
<td>L18</td>
</tr>
<tr>
<td>PEG</td>
<td>85&lt;sup&gt;57&lt;/sup&gt;</td>
<td>L19</td>
</tr>
<tr>
<td>PMVE</td>
<td>35&lt;sup&gt;58&lt;/sup&gt;</td>
<td>L20</td>
</tr>
<tr>
<td>POEGMA</td>
<td>26-90&lt;sup&gt;55, 59-60&lt;/sup&gt;</td>
<td>L21</td>
</tr>
<tr>
<td>PDEAEMA</td>
<td>14-50&lt;sup&gt;61&lt;/sup&gt;</td>
<td>L22</td>
</tr>
<tr>
<td>PDMAEMA</td>
<td>&lt; 0&lt;sup&gt;62&lt;/sup&gt;</td>
<td>L23</td>
</tr>
<tr>
<td>CEs</td>
<td>4-33&lt;sup&gt;63&lt;/sup&gt;</td>
<td>L24</td>
</tr>
<tr>
<td>PVCa</td>
<td>32&lt;sup&gt;64&lt;/sup&gt;</td>
<td>L25</td>
</tr>
</tbody>
</table>
2.3.1 Poly(N-substituted acrylamide)s

Poly(N-substituted acrylamide)s (PAMs, L1 – 10) stand as the most frequently studied aqueous thermoresponsive polymers. The synthesis of acrylamides was originally reported by Specht in 1956, with the first reports of PAMs being used as rodent repellants by Eastman Kodak. Eight years after polymer LCST behavior was first reported in literature, Heskins and Guillet published work detailing PNIPAM aqueous LCST phase diagrams. Over the course of 50 years, PNIPAM has since become the “gold standard” of thermoresponsive polymers for biomedical applications due in large part to the sharp aqueous phase transition occurring near 32 °C, above typical ambient temperatures but below body temperature of 37 °C. Furthermore, PNIPAM displays a rather robust phase transition that is not heavily influenced by solution concentration, pH, osmolarity, and molecular weight. Many excellent reviews exist which explore the thermoresponsivity of PNIPAM alone. \(^1, 3-4\)

PNIPAM also benefits from the ability to be copolymerized with a variety of monomers in order to tune its thermal and physical properties. \(^67-68\) By using controlled polymerization techniques such as Atom Transfer Radical Polymerization (ATRP) and Reversible Addition-Fragmentation chain Transfer (RAFT), complex architectures such as blocks, combs, and grafts can be created. In particular, PNIPAM block copolymers containing hydrophobic or pH sensitive monomers have garnered significant interest as thermoresponsive micelles\(^4, 68\) and injectable gels\(^69\) for drug delivery (Figure 2.7). \(^70\) Due to PNIPAMs similar structure to the poly(amide) backbone of proteins, PNIPAM has been used as model to explore mechanism of protein denaturation by urea. \(^22, 71-73\)
PNIPAM is not without disadvantages. The high glass transition temperature \( T_g \) of PNIPAM (140 °C) results in the formation of glassy material as the polymer dehydrates and becomes concentrated. The presence of a hydrogen bond donor and acceptor in the backbone generates many intra- and intermolecular hydrogen bonds in the dehydrated polymer globule. Breaking and replacing these bonds by bulk water prevent rapid re-solvation of the polymer and lead to significant hysteresis.\(^{74-75}\) The phase transition temperature of low molecular weight PNIPAM is considerably affected by the end group hydrophobicity.\(^{76-78}\) Inherent to all PAMs, the carbon backbone prevents biodegradation which is limiting for numerous biological applications. The significant dehydration of PNIPAM above the LCST leads to drastic volume shrinkage, which can result in the rapid release of encapsulated compounds and mechanical stresses on entrapped biomolecules (leading to loss of structure and function) and cells (leading to cell death).\(^8, 79\) Additionally, questions have been raised about the possible cytotoxicity of PNIPAM.\(^{80}\)

By modifying the \( N \)-substitution, PAMs displaying a variety of phase transition temperatures have been explored. In general, the LCST follows the substituent hydrophobicity: increasingly hydrophobic substituents decrease the LCST while more hydrophilic
substituents serve to raise it. For example, changing the \(i\)-propyl group to more hydrophobic \(n\)-propyl group results in the LCST of poly(\(N\)-\(n\)-propylacrylamide) (PNNPAM, L3) dropping to 23 °C. Poly(\(N\)-\(c\)-propylacrylamide) (PNCPAM, L4), although having a similar structure to PNIPAM displays a LCST at 53 °C. This can be explained using Saito’s model of the previously described hydrophobic effect, which puts forward that the surface area presented by the alkyl substituent affects hydrophobic hydration.\(^{67}\) Substituents like the \(c\)-propyl group, which has a smaller surface area than the \(i\)-propyl group, interact with fewer water molecules in the hydrophobic hydration shell leading to an increase in the LCST.

Generally, any substituents on PAMs bearing more than four carbons do not exhibit LCST behavior unless polar heteroatoms or groups are included. For example, substituting polar oxygen atoms for methylene groups gives rise to poly(\(N\)-alkoxyacrylamide)s (PAOAM, L6 – 10).\(^{46-49, 81}\) PAOAMs typically show much higher phase transition temperatures than their PAM analogues which offers an interesting route for tuning of the thermal response. For instance, while \(n\)-butyl substituted PAM is too hydrophobic to be water soluble, hydrophilic methoxyethyl substituted PMoEA (L8) is completely water soluble and does not exhibit a LCST. Adding an additional methylene gives rise to slightly more hydrophobic ethoxyethyl substituted PEoEA (L7) with an LCST near body temperature at 38 °C. The effects and trends of numerous alkoxy substituents on the resultant temperature driven phase separation of PAOAMs has been reported in a detailed study by Ito.\(^{48}\) The incorporation of tertiary amide groups, such as with PDMAEMA (L22), offers a unique route for tuning the stimuli-response. Not only do the polar amines and their substituents modify the temperature response in neutral water, but they are also subject to pH-dependent ionization.\(^{41}\) Additionally, it has recently been shown that tertiary amine substituents are
CO₂ responsive as well, making tertiary amine groups attractive for generating temperature, pH, and CO₂ triple-responsive materials.\textsuperscript{82-84}

When heated above the LCST, some PAMs have been shown to undergo coacervation (discussed in further detail in 2.3.3), a liquid-liquid phase separation into a polymer-rich and polymer-poor phase. This seems to require the presence of an extremely hydrophilic substituent such as dimethylamide,\textsuperscript{85-86} hydroxyl,\textsuperscript{5-7, 87-88} carboxylic acid,\textsuperscript{7-8} or numerous ether bonds.\textsuperscript{89-92} These hydrophilic substituents must be balanced by an appropriate level of overall hydrophobic character in order for an LCST to be observed. The coacervation-driven temperature response suggest that extremely hydrophilic nature seems to prevent complete dehydration from taking place above the LCST.

2.3.2 Poly(oxazoline)s

After PAMs, one of the best studied thermoresponsive polymers is that of poly(2-alkyl-2-oxazoline)s (PAOxs, L11 – 16). The cationic ring-opening polymerization (CROP) of PAOxs was first reported by four independent research groups in 1966.\textsuperscript{93-96} CROP is still the most common method for the synthesis of PAOx, with 2-oxazoline monomers generally used as unfavorable steric make polymerization of the 4- or 5-substituted position difficult. The resultant polymers are structural analogues of proteins and PAMs, so it is logical to assume that certain PAOx may display temperature dependent phase behavior (Scheme 2.1). This was first reported by Aponte and coworkers for poly(2-ethyl-2-oxazoline) (PEtOx, L12) in 1988.\textsuperscript{51}
Scheme 2.1: Polymerization of 2-oxazolines as well as the structural analogy with poly(amino acid)s and PAMs.37

Similar to PAMs, the thermal sensitivity of PAOx is governed primarily by the hydrophobicity of the pendant group (Figure 2.8). Poly(2-methyl-2-oxazoline) (PMeOx, L11) is completely water soluble while the homopolymer LCST decreases from 60 °C > 38 °C > 25 °C as the pendant groups are replaced with increasingly more hydrophobic ethyl (PEtOx), i-propyl (PiPrOx, L13), and n-propyl (PnPrOx, L15) groups. It is interesting to note that the c-propyl group (PcPrOx, L14, LCST ~ 30 °C) seems to exhibit less of a hydrophobic effect than is observed with PAMs. The phase transition temperature can be tuned via copolymerization with monomers of varying hydrophobicity97 as well as through post-polymerization modification. The effects of end group hydrophobicity and solution osmolarity are similar. Thermosensitive gels,98-101 and micelles102-104 have been prepared and explored as promising means of triggered drug delivery. However, as is the case with PAMs, the non-degradable backbone of PAOx materials can be limiting for certain applications.
Figure 2.8: The homologue series of PAOxs that share the polar amide motif with decreasing water solubility as the 2-alkyl side chain length increases. PAOxs with intermediate side chain length display a temperature dependent solubility. Reprinted with permission from Elsevier, 2010.

As compared to PAMs, PAOxs exhibit certain differences regarding LCST behavior. First, the LCST of PAOxs are much more influenced by molecular weight and concentration than is observed with PAMs. Very little thermal hysteresis is observed for PAOxs which is ascribed to the lack of a hydrogen bond donor as well as lower \( T_g \) as compared to PAMs. When held above the LCST for prolonged periods of time, some PAOxs, such as PiPrOx, will begin to crystallize which prevents reversibility of the phase separation. Interestingly, crystalized PAOx nanoparticles have been shown to order into structured nanofibers.

PAOx materials are relatively non-toxic and as such have been widely studied for biomedical applications for the past 25 years. Another reason for the popularity of PAOx biomaterials can be attributed to the observation that some PAOxs, such as PPMeOx and PEtOx, exhibit “stealth” behavior within biological systems. This is likely due to the structural similarity between PAOx and the most well-studied “stealth” polymer, poly(ethylene glycol) (PEG, L19). Since the early 1990s, materials coated with PEG have been shown to minimize protein binding (opsonization) by the mononuclear phagocyte system (MPS),
possibly as a result of PEG chain flexibility and hydrophilicity. This prevents recogni-
tion by the MPS and allows the biomaterial to avoid immune system response and clear-
ance for longer periods of time.\textsuperscript{109-111}

2.3.3 Elastin-like Peptides

Elastin is a naturally occurring elastic extracellular matrix (ECM) protein com-
monly found in connective tissues; the flexibility of elastin is the reason why pinched skin
easily returns to its original shape. Tropoelastin is a water-soluble protein that serves as the
precursor to elastin. The structure of tropoelastin is comprised of significant amounts of
alternating hydrophobic and hydrophilic (mostly lysine-containing) domains. This results
in the formation of a protein-rich, water-poor coacervate phase amid the surrounding aque-
ous (water-rich, protein-poor) phase immediately after synthesis.\textsuperscript{112-113} Directly after the
completion of synthesis and coacervation, the lysine residues on tropoelastin are enzymat-
ically crosslinked to generate fresh elastin.\textsuperscript{114-115}

Elastin-like polypeptides (ELPs, L17) are artificial polypeptides that earn their
name from the sequence of five amino acids that make up a commonly repeated sequence
in the hydrophobic domains of tropoelastin: \((VPGXG)_n\) where \(X\) is any amino acid other
than proline and \(n\) is the number of sequence repeats.

First reported by Urry and coworkers in 1986,\textsuperscript{116} above a critical temperature ELPs
undergo a sharp phase transition marked by a sudden increase in solution turbidity, much
like the \(T_{cp}\) observed in other LCST-polymers. The temperature-driven phase separation of
synthetic ELPs can be very sharp (\(\sim 3\) °C) and is commonly referred to as the inverse tran-
sition temperature \((T_t)\) instead of the LCST or \(T_{cp}\). The difference in nomenclature is due
to a number of distinctions between ELPs and other synthetic LCST-type polymers: ELPs
are typically slightly charged at neutral pH, their demixing behavior is much more influenced by changes in pH and salt, and is heavily influenced by solution concentration. Additionally, upon dehydration the ELP often folds into complex structures such as β-sheets and β-spirals/turns.

Similar to other LCST-type materials, the $T_c$ of ELPs depends on the overall balance of hydrophobic and hydrophilic domains within the polymer. Despite short hydrophobic and hydrophilic regions being scattered along the backbone and side chains, the overall mean hydrophobicity of the guest residue $X$ allows for simple tuning according to sequence and desired chain length. Over the past 30 years, the versatility of ELPs has made them attractive candidates for recombinant protein purification, drug delivery as nanocarriers, micelles, and local delivery depots, and as substrates for tissue engineering.

Is it important to note that the temperature-driven dehydration mechanism of ELPs is different than for most LCST-type polymers. Not all temperature-responsive polymers undergo efficient dehydration and subsequent coil–globule transition such as exhibited by PNIPAM. Instead, some polymers, such as ELPs, undergo an incomplete dehydration when brought above the LCST. The partially dehydrated polymers then separate into polymer-rich coacervate droplets within a polymer-deficient liquid phase, comparable to the coacervation observed with tropoelastin. Coacervate-type polymers are particularly attractive from a biomaterials standpoint since their incomplete dehydration leads to minimal conformational change as compared to coil–globule polymers. This prevents coacervate-type polymers from damaging sensitive biomolecules and thus allows them to be used as agents for the purification of proteins and nucleic acid without disrupting their function.
as controlled delivery agents for sensitive physiologically active molecules,\textsuperscript{123} and as injectable scaffolds.\textsuperscript{55, 127} Despite this advantage, there have been far fewer reports in the literature on thermoresponsive coacervate-type polymers as compared to coil–globule type polymers and ELPs remain the most common example of materials which display this behavior.

In addition to the unique temperature-driven dehydration mechanism of ELPs, synthetic polypeptides offer a number of advantages as compared to synthetic LCST-type polymers. Since polypeptides can be programmed at the genetic level, it is possible to synthesize monodisperse polymers with complete control over sequence. ELPs are comprised of amino acids linked by hydrolysable amide bonds which limits the toxicity of the polypeptides and their degradation products. Probably the most significant limitation to ELPs is the molecular biology techniques required for synthesis, such as recursive directional ligation and recombinant synthesis.\textsuperscript{128} Additional limitations are the relatively short shelf life, thermal instabilities, and design constraints imposed by primarily 20 naturally occurring (and some recent studies on unnaturally occurring) amino acids.\textsuperscript{129}

2.4 Synthetic, Biodegradable Polymers

Polymeric biomaterials can be classified according to their stability in a biological system: biostable, partially biodegradable, and degradeable.\textsuperscript{130} Biostable polymers, such as poly(ethylene) and poly(methyl methacrylate), do not evoke a significant immune system response and do not undergo degradation in living tissue. Many biostable polymers can last for years, making them attractive for long-lasting implants such as sutures and joint prostheses.
Conversely, biodegradable polymers are attractive for numerous medical applications, especially within the realm of tissue engineering and drug delivery. Polymeric degradation serves as a safe and convenient means for removal of a biomaterial from the host. This can eliminate the need for invasive surgical removal which runs the risk of infection in addition to other surgical complications. Additionally, degradation lessens concerns regarding continued biological activity/impact after timeframe for therapeutic use, such as the formation of a fibrous capsule around non-native implanted materials. Degradation reduces the need for “excellent material biocompatibility,” as compared to biostable polymers, since the degradable materials are not long-lasting in the body. Importantly, degradation can be used as a mechanism by which to release therapeutics, imaging compounds, genes, proteins, and cells at a controlled rate from constructs, microspheres, and nanoparticles. Excellent reviews by Laurencin and Kasko highlight many of the recent advances and strategies for polymeric biomaterial degradation.

It is not difficult to imagine a variety of next-generation medical applications for thermo-responsive, biodegradable polymers. Solutions containing cells, biologic factors, and polymer could be injected to create permeable scaffolds at body temperature. Such a delivery system would eliminate the risks of surgical implantation while creating a scaffold uniquely shaped to the injected area. Polymer nanoparticles loaded with therapeutics could display different release profiles: slowly during degradation or rapidly at locally cooled environments. Liquid bandages comprised of polymer and antimicrobials might be applied to completely seal intricate wounds in a matter of seconds, degrading as tissue heals. However, biodegradation is not possible for the most common synthetic thermo-responsive pol-
ymer, such as PAMs and POXs, due to their non-degradable backbone. While ELPs possess inherent degradability due to hydrolysis and possible enzymatic cleavage of the amide backbone, they suffer from slow degradation rate (often requiring proteases) and a complex synthetic route. As such, research into new synthetic biodegradable and thermoresponsive materials has become an area of increased interest.

While there are many strategies by which polymeric degradation can be achieved, such as enzymolysis, redox, UV, magnetic field, and ultrasound, hydrolysis remains one of the most attractive due to its simplicity and availability in biological environments (Figure 2.10). Since the first reported use in 1967, the most common surgically implanted biodegradable polymers are aliphatic poly(ester)s (PEs) such as Dexon® and Vicryl®. A common strategy to achieve hydrolytically partially degradable polymers involves the copolymerization of a non-degradable monomer with a comonomer capable of undergoing hydrolytic degradation. This approach has been used to synthesize biodegradable block copolymer micelles for drug delivery, as well as for biodegradable gels. Ameer and coworkers recently utilized this method to design a thermoresponsive partially biodegradable gel with antioxidant properties for wound healing applications. By functionalizing citric acid with PEG and acrylate moieties, radical polymerization with NIPAM resulted in a low molecular weight branched poly(polyethylene glycol citrate-co- N-isopropylacrylamide) (PPCN) copolymer that gelled at 26 °C in PBS buffer with minimal gel shrinkage (Figure 2.9). The formed PPCN gels showed a 25% mass loss after 2 months in vivo stemming from hydrolytic degradation of ester bonds.
Figure 2.9: Synthesis of PPCN copolymer and formation of branched, antioxidant PPCN gels. Reprinted with permission from The American Chemical Society, 2014.

Although materials with degradable characteristics generated through the copolymerization strategy are promising for a number of applications, the sections of material displaying inherent non-degradability must be of low enough molecular weight to be cleared by the renal system. Ideally, the entire material would degrade to low molecular weight byproducts within a desirable timeframe. For the purpose of this publication, only thermoresponsive materials able to undergo complete degradation will be discussed further.
In order to create a thermoresponsive biomaterial with engineered total hydrolytic degradation, a number of factors must be taken into account. Most importantly, the degradation products must exhibit low toxicity. Hydrolytic sites such as ethers and amides must be inserted into the polymer backbone at each repeat unit. The rate of degradation must also be taken into account. Generally, hydrolysis is affected by a number of molecular and structural factors. Lower stability bonds, such as ethers, are more prone to hydrolysis than more stable bonds such as amides and carbonates. Increased $T_g$ generally correlates to decreased polymer mobility, leading to a reduction in the rate hydrolysis. Increasing molecular weight for a given polymer typically increases the degradation time. Modifying the solution pH to acidic or basic conditions is often explored to mimic biological conditions that will affect the rate of hydrolysis. 

Table 2.2). This can increase polymer degradation depending on the type of hydrolytic bond, with bonds producing acidic byproducts resulting in hydrolytic autocatalysis.
Table 2.2: pH in various biological tissues.\textsuperscript{30,134}

<table>
<thead>
<tr>
<th>Tissue and Cell Compartments</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>7.4</td>
</tr>
<tr>
<td>Tumor extracellular environment</td>
<td>6.5 – 7.2</td>
</tr>
<tr>
<td>Endosome</td>
<td>5.0 – 6.5</td>
</tr>
<tr>
<td>Lysosome</td>
<td>4.5 – 5.0</td>
</tr>
<tr>
<td>Colon</td>
<td>7.0 – 7.5</td>
</tr>
<tr>
<td>Intestine</td>
<td>5.0 – 8.0</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.0 – 3.0</td>
</tr>
</tbody>
</table>

Even after taking degradation considerations into account, the prediction of possible thermosensitivity is more difficult as compared to non-degradable PAMs or PAOxs. The degree of hydrophilicity inherent to the hydrolytic site, the frequency at which the sites appear along the backbone, the hydrophobicity of the regions between hydrolytic sites, and the hydrophobicity of any pendant groups must act cooperatively to create a beneficial hydrophobic/hydrophilic balance. To date, no de novo equation or model exists that can predict whether or not a proposed novel polymer will exhibit temperature driven phase separation. Most studies rely on slight chemical modifications to existing biodegradable thermoresponsive polymers.

2.5 Examples of synthetic biodegradable LCST-type polymers

As previously discussed, ELPs can be considered thermoresponsive biopolymers that are capable of biodegradation. However, the practical limitations of ELPs and the incomplete degradation of other currently available materials can only be solved through the design of novel synthetic polymers containing inherent degradable sites. Biodegradable sites are typically hydrophilic, such as esters and amides, which require a hydrophobic
balance in the side chain to impart a temperature driven response. To date, only a few synthetic thermoresponsive polymers with controlled degradation have been reported, each with specific advantages and disadvantages and no material standing out as “ideal.”

The difficulty of designing new materials capable of thermosensitivity and complete degradation was highlighted in a recent report by Koberstein and coworkers in *Macromolecules*. Using step-growth polymerization between divinyl oligo ethylene glycol (OEG) and an OEG diol, a library of poly(acetal)s (L26) with tunable $T_{cp}$ were successfully synthesized. The poly(acetal)s degraded to neutral byproducts under acidic conditions. While the highly tunable thermoresponse (6 – 80 °C) and lack of acidic byproducts are worth note (especially for biomedical applications), a primary goal of the study was to design a material with inherent biodegradability “…because synthetic thermoresponsive polymers developed to date lack an intrinsic biodegradation mechanism.” In fact, two years prior Aoshima and coworkers reported in *ACS Macro Letters* the synthesis of poly(acetal)s (L27) with predicted tunable thermosensitivity (13 – 86 °C), acid-catalyzed hydrolysis, and neutral byproducts.

Perhaps the most widely used strategy to design novel thermoresponsive biodegradable materials involves incorporating PEG (L19) or OEG into hydrophobic, biodegradable polymers. PEG/OEG chains are very hydrophilic but exhibit sharp LCST behavior that can be tuned via chain length (L21, 30 – 31, 33 – 39). Incorporating PEG/OEG into otherwise hydrophobic degradable polymers, either as side chains or in the main chain, will often yield a thermoresponsive material.
Figure 2.11: Select degradable thermoresponsive homo- and copolymers. Hydrolytically cleavable bonds are shown in red, LCST or $T_{cp}$ (°C) are shown in parentheses.
PEs and poly(carbonate)s (PCs) represent a unique perspective as biodegradable materials and are likely the most studied candidates for thermoresponsive biodegradable polymers. While the ester bond is hydrolysable, the rate of hydrolysis can vary from days to months depending on a number of previously discussed factors such as hydrophobicity, $T_g$, and crystallinity.\textsuperscript{132} Both types of polymers have been explored as thermoresponsive biodegradable polymers and are typically synthesized via ring-opening polymerization (ROP) of OEG-functionalized ethylene oxide (L30),\textsuperscript{151} lactides (L32),\textsuperscript{152} lactones (L33 – 35),\textsuperscript{153} and cyclic carbonates (L39).\textsuperscript{153} Another strategy involves post-polymerization functionalization of the PEs and PCs with OEG.\textsuperscript{154} Recently, Wang and coworkers have published a number of reports detailing the copolymerization of CO$_2$ and ethylene oxide to generate highly tunable thermoresponsive PCs (L36 – 38).\textsuperscript{155-157} A limitation of most thermoresponsive PEs and PCs is their lack of orthogonal functionality and reported degradation studies, specifically with PCs.

Another category of well-studied synthetic thermoresponsive biodegradable polymers are poly(phosphoester)s (PPEs). Synthetic PPEs are analogues to the naturally occurring PPE backbone of DNA and RNA and are relatively stable in neutral water. The ready cleavage of the phosphoester backbone at accelerated rates under acidic and basic conditions and by enzymes such as alkaline phosphatase makes them attractive candidates for biomedical applications.\textsuperscript{158} Since the first reports by Penczek and coworkers in the late 1970s, PPEs synthesized via CROP\textsuperscript{159-160} and polycondensation\textsuperscript{161-162} have been investigated for drug delivery. Interestingly, it was not until 2007 that Iwansaki and coworkers reported that poly(ethyl ethylene phosphate) (PEEP, L28) exhibited a highly reversible $T_{r,p}$ at 40 °C.\textsuperscript{163} Random copolymerization with more hydrophobic $i$-propyl ethylene phosphate
(IPP, L29) led to a linear decrease of $T_{cp}$ proportional to IPP feed ratio, which allowed for tunability of the thermal response. Similar to other thermoresponsive materials, the phase transition temperature of PPEs was observed to be slightly inversely proportional to molecular weight as well as NaCl concentration. Biodegradable micelles comprised of a thermoresponsive PPE block and either a hydrophobic poly(e-caprolactone) or hydrophilic PEG block were shown to exhibit low cytotoxicity and have been explored for possible use as targeted drug delivery vehicles. Iwansaki later expanded the thermoresponsive PPE library to include copolymers bearing enzyme-cleavable side chains, useful for improving and tuning polymer solubility.

However, since the publication of these initial studies, interest in thermoresponsive PPEs seems to have diminished: only five reports of thermoresponsive PPEs have been published since 2009 according to SciFinder (searching the terms “LCST polyphosphoester” and “thermoresponsive polyphosphoester”). One notable exception is a recent study by Wooley and Dove in which PPE-b-poly(D-glucose carbonate) (PDGC) block copolymer micelles were shown to degrade into natural byproducts. The lack of recent interest may be due to the lack of functionality, a similar drawback for many thermoresponsive biodegradable PEs and PCs, as well as a lack of thermoresponsive monomer diversity. However, PPEs have recently been described as exhibiting temperature-driven coacervation similar to what is observed for ELPs which may make them more attractive for biomedical applications.

A number of polyamides (PAs, L40 – 44) with tunable LCST have been proposed as biodegradable thermoresponsive polymers. Examples include poly[α,β-(dl-asparte i-
propylamide)-co-(succinimide), poly(α-substituted-L-glutamate)s, poly(aspartamide) and poly(asparagine) derivatives, poly(N-substituted-L-glycine)s also known as poly(peptoid)s. Unfortunately, many of the best studied PAs require multiple difficult synthetic steps suffering from low yield to obtain monomer. The PA molecular weights are generally low. Almost all reported biodegradable thermoresponsive PAs lack degradation studies of any kind. Similarly, an interesting recent report by Hedrick and Yang on biodegradable, thermoresponsive poly(ether urethane)s (L45) also omitted degradation experiments. In each case, the authors note the established hydrolytic degradation of the incorporated degradable amide bond. However, since the overall polymer structure is highly influential on the type and rate of degradation, preliminary degradation data for each novel polymer would have been incredibly useful.

While it is unlikely that there will ever be a universal “ideal” thermoresponsive biodegradable polymer for all biomedical applications, addressing the limitations of many extant polymer systems is critical in designing a novel material that could find widespread use. Virtually all the polymers addressed lack functionality other than is used for post-polymerization attachment of OEG. This lack of functionalization may be limiting if covalent attachment of therapeutics/imaging agents or complex interaction with the surrounding environment is desired. Low $T_g$ is desirable. Additionally, many of the materials rely on slow hydrolysis whereas a more quickly degradable material may be useful for certain applications. Finally, there is a general lack of monomer variety in many of the previously described systems, limiting the tunability of physical, thermal, and solution properties.
2.6 Carbodiimide-mediated Polyesterification

As previously discussed, PEs are attractive candidates for designing novel thermoresponsive biomaterials due to the inherent biodegradability of the ester backbone. Most synthetic PEs are synthesized using one of two methods: polycondensation of a hydroxy-acid or diacid and diol, or by ROP of a lactide.\textsuperscript{178-180}

The current popular synthetic approaches can be used to generate a vast number of homo- and copoly(ester)s, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactide-co-glycolic acid) (PLGA), poly(caprolactone). The degradability of these materials is dependent on a number of factors such as $T_g$, molecular weight, crystallinity, hydrophobicity, and porosity. Recent studies by a number of researchers have focused on methods to introduce functionality into polyesters in order better interact with biological systems. The most heavily studied strategy involves ROP of novel functionalized lactides. For example, Hedrick and coworkers utilized ROP of protected $\varepsilon$-caprolactone derivatives and subsequent deprotection to generate a library of functionalized aliphatic PEs with narrow polydispersities (Scheme 2.2).\textsuperscript{181}
Scheme 2.2: Polymerization and subsequent deprotection of functionalized ε-caprolactone monomers.\textsuperscript{181}

To date, there exist far fewer reports in literature describing polycondensation of functionalized diol or diacid monomers to generate functionalized polyesters. This is mostly due to the harsh reaction temperatures required by traditional polycondensations which remove water and drive the reaction forward by Le Châtelier's principle. These conditions are not tolerant of sensitive functional groups and invite the possibility of undesirable side-reactions.\textsuperscript{182} Furthermore, PEs synthesized by high-temperature polycondensations...
tion typically suffer from low molecular weight and high polydispersity ($D_M$) due to transesterification, in stark contrast to the controlled molecular weights of polyesters synthesized by ROP.

Esterification of small molecules has also been described using coupling agents such as carbodiimides. Carbodiimides, such as dicyclohexylcarbodiimide (DCC) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), are commonly used in peptide synthesis to form amides by coupling amides to carboxylic acids. In 1978, Steglich first reported room-temperature esterification using a DCC coupling agent and 4-dimethylaminopyridine (DMAP) as a catalyst in what is now referred to as the Steglich esterification. Water is chemically, as opposed to physically, removed from the reaction as a stable N-acylurea byproduct for each ester bond formed (Scheme 2.3).

Scheme 2.3: The Steglich esterification using DCC and DMAP.

Utilizing a modified version of the Steglich esterification, Moore and Stupp first reported the use of room-temperature carbodiimide-mediated coupling chemistry to synthesize high molecular weight ($DP_n > 50$) semi-aromatic polyesters from self-condensation of a hydroxy acid (Scheme 2.4): 

Scheme 2.4: Polyesterification of a semi-aromatic hydroxy acid via room=temperature DIC/DPTS coupling.
Moore and Stupp made a number of improvements to allow for the generation of high molecular weight material in their highly cited report. First, $N,N'$-diisopropylcarbodiimide (DIC) was utilized as a coupling agent instead of DCC. DIC is a less potent allergen than DCC and is easier to handle as DIC is a liquid while DCC is a waxy solid. Additionally, the $N,N'$-diisopropylurea byproduct is easier to remove and trace amounts have been shown to be non-toxic.\(^{185}\) The catalyst was changed to a salt of DMAP and p-toluenesulfonic acid (pTsOH), 4-(dimethylamino) pyridinium-4-toluene sulfonate (DPTS). It was found that an equimolar ratio of DMAP : pTsOH suppresses the formation of carboxylic acid groups to unreactive $N$-acylurea which prevents the formation of high molecular weight material. The polymerization did not result in $N$-acylurea end groups on the PEs. Furthermore, this method is tolerant to trace amounts of water as the resulting reaction will simply regenerate a carboxylic acid capable of undergoing esterification.\(^{186}\)

In order to combat the drawbacks of traditional high-temperature polyesterification, room-temperature carbodiimide-mediated esterification has recently been gaining interest as a route to synthesize high molecular weight, PEs with narrow $D_M$ and biologically useful functionalities not accessible through other polyesterification methods. Examples include smart materials that respond to stress\(^{187-188}\) and light,\(^{189-190}\) alternating copolymers,\(^{191-193}\) hyperbranched\(^{194-196}\) and dendritic materials,\(^{197-200}\) and hydrophilic materials bearing structural similarities to polypeptides.\(^9\)
CHAPTER III

EXPERIMENTAL METHODS

3.1 Materials

All chemicals were used as received unless otherwise indicated. Methyl butyrate (98%), methyl isobutyrate (98%), methyl methoxyacetate (99%), ethyl methoxyacetate (97%), ethyl succinyl chloride (98%), isopropylamine (99%), pyrrolidine (99%), propylamine (98%), cyclopropylamine (99%), diethylamine (99%), triethylamine (Et3N, 98%), morpholine (99%), di-n-butylamine (98%), heptylamine (98%), diethanolamine (DEA, 99%), 4-isobutyl-alpha-methylphenylacetic acid (ibuprofen, 99%), sodium chloride (NaCl, 99%), sodium nitrate (NaNO3, 98%), sodium bromide (NaBr, 97%), sodium iodide (NaI, 98%), sodium fluoride (NaF, 99%), sodium sulfate (Na2SO4, 99%), sodium dihydrogen phosphate monohydrate (NaH2PO4•H2O, 98%), sodium thiosulfate pentahydrate (Na2S2O3•5H2O 99%), urea (98%), and sodium dodecyl sulfate (SDS, 98%) were purchased from Acros Organic. Bis(2-methoxyethyl)amine (98%), 2-methoxyethylamine (98%), tetrahydrofurfurylamine (98%), and boc-L-glutamic acid (99%) were purchased from TCI America. N,N'-diisopropylcarbodiimide (DIC, 99%) was purchased from Oakwood Chemical. Piperidine (99%) was purchased from Chem Impex International. γ-butyrolactone (99%) was purchased from Sigma Aldrich. Succinic acid (99%) was purchased from Alfa Aesar and recrystallized from water. 4-(dimethylamino) pyridinium 4-toluene sulfonate
(DPTS) was prepared according to literature methods.\textsuperscript{201-202} Reagent grade dichloromethane (CH$_2$Cl$_2$) was purchased from Thermo Fisher Scientific and dried by distilling over anhydrous CaH$_2$. Reagent grade ethyl acetate (EtOAc), tetrahydrofuran (THF), and methanol (MeOH) were used as received from Thermo Fisher Scientific. Silica gel (40-63 µm, 230 x 400 mesh) for flash chromatography was purchased from Sorbent Technologies, Inc. Dialysis tubing (regenerated cellulose, MWCO 3500 Da) was obtained from Thermo Fisher Scientific. Deionized water (DI H$_2$O) was used to prepare polymer solutions unless otherwise stated.

3.2 Techniques

Procedures for analysis of monomer and polymer purity, characterization of physical and solution properties, as well as biological studies are detailed below.

3.2.1 Nuclear magnetic resonance spectroscopy (NMR)

All $^1$H and $^{13}$C NMR spectra in CDCl$_3$ of the monomers and polyesters were recorded on either a Varian Mercury 300 MHz or 500 MHz spectrometer. Chemical shifts were recorded in ppm (δ) relative to solvent signals. Variable temperature $^1$H NMR spectra in D$_2$O were recorded on a Varian INOVA 400 MHz spectrometer with 15 min equilibrations at each temperature.

3.2.2 Differential scanning calorimetry (DSC)

Glass transition temperatures ($T_g$) of the polymers were determined using a TA Q2000 DSC with a liquid N$_2$ cooling unit and a heating/cooling rate of 10 °C/min.
3.2.3 Size exclusion chromatography (SEC)

Polymer molecular weights were analyzed on a TOSOH EcoSec HLC-8320 SEC equipped with a refractive index detector (RI) and UV detector. Separation occurred over two PSS Gram Analytical SEC Columns in series using 25 mM LiBr in DMF as eluent at a flow rate of 0.8 mL/min. The column and detector temperatures were maintained at 50 °C. Molecular weights were obtained relative to PMMA standards using the RI signal. Optical microscopy was carried out on an Olympus IX81 Motorized Inverted Microscope using either Brightfield or TRITC channel filters.

3.2.4 Ultraviolet-visible (UV-vis) spectroscopy

Turbidity measurements were carried out on a Shimadzu UV-1800 UV–vis spectrophotometer equipped with a Shimadzu S-1700 thermoelectric single cell holder in a 1 cm quartz cell. Deionized water was used as a reference. Polymer solutions (10 mg/mL unless otherwise noted) were prepared in DI water and left at 4 °C overnight to ensure complete dissolution and equilibration. Solutions were equilibrated at 0 °C or 5 °C until no change in transmittance was observed. Transmittance was recorded as a function of temperature at 1 °C/min and a fixed 350 nm wavelength. The cloud point temperature ($T_{cp}$) for a particular experiment was defined as the temperature at which the transmittance was 50%.

3.2.5 Dynamic light scattering (DLS)

DLS measurements of the homopolymer aqueous polymer solutions (0.5 mg/mL) were performed on a Brookhaven Inc. Laser light scattering spectrometer equipped with a
temperature controlled and a solid state laser ($\lambda = 532$ nm, detection angle: $90^\circ$). An intensity-intensity time correlation function was measured by means of a multichannel digital correlator, which was then processed using the CONTIN method to obtain the average hydrodynamic radius of the particles in solution. The solutions were filtered through a $0.45 \, \mu$m PVDF filter prior to measurements. The solutions were equilibrated for 30 min at each temperature. Increasing the temperature far above the $T_{cp}$ over the long duration of the experiment resulted in very turbid solutions that did not allow the laser of the Brookhaven Goniometer to fully transmit, as such experiments were only carried out while accurate scattering data could be obtained.

3.2.6 Cell viability

The effect of polymer on the viability of mammalian cells was performed using NIH-3T3 mouse embryonic fibroblasts (kindly donated by the Becker Lab). The NIH-3T3 cells were cultured using a growth medium composed of DMEM supplemented with 10% fetal bovine serum (Hyclone) and 1% penicillin-streptomycin (10,000 U/mL, Thermofisher Scientific) at 37 °C in a 5% CO$_2$ environment. For the experiment, the cells were expanded to ~75-85% confluence, harvested, and then seeded into a tissue culture 96 well plate at a density of ~5,000 cells/cm$^2$. After allowing the cells to adhere and grow overnight, the medium was replaced with medium that was supplemented with the desired polymer. The growth media containing polymer was prepared by first making multiple concentrations of the polymer in PBS by performing a serial dilution of a concentrated polymer stock solution (10 mg/mL) to make multiple concentrations of the polymer solution in PBS. Then, using the stock solution and the serially diluted solutions of polymer in PBS, growth media
supplemented with 10% of the solutions were prepared. CellTiter-Blue® cell viability assay (Promega) was performed after allowing the cells to incubate for one day in the polymer supplemented media. The assay was performed by aspirating the medium from each well, rinsing the cells with warm PBS solution, and then adding growth medium supplemented with 20% of CellTiter-Blue® reagent. Once the reagent was added, the well plate was incubated for 1 hr followed by fluorescence measurement at 560 nm/590 nm for each well on a Biotek Synergy 2 multimode microplate reader. Each concentration was tested using 6 replicates.

3.2.7 Qualitative and Quantitative Analysis of LCST Polymer Ability to Encapsulate FITC-BSA

All polymer solutions were prepared by dissolving the polymers to a concentration of 10mg/mL in 100 mM phosphate buffer prepared to either pH 6.0, 7.0 or 8.0 and allowing to sit in a ~4 °C refrigerator overnight before use. FITC-BSA was prepared by dissolving to a concentration of 1 mg/mL or 0.1 mg/mL in the same phosphate buffers used for the polymers. Once prepared, the solutions of polymers and proteins were stored at -80 °C and thawed for use when needed. Each solution was subjected to no more than three freeze thaw cycles. For both microscopy and assay, the encapsulation was performed by adding the polymer and FITC-BSA in a one to one ratio to prepare solutions that contained 5 mg/mL polymer and 0.5 mg/mL FITC-BSA (for microscopy) as well as solutions that contained 5mg/mL polymer and 0.05 mg/mL FITC-BSA (for assay). After combining each component, the polymer/FITC-BSA mixtures were vortexed, placed into a ~4 °C refrigerator for 30 min, vortexed again, and incubated at 37 °C for 30 min. For microscopy, the formed coacervates were vortexed and one drop of the solution placed onto an untreated
microscope slide. Imaging of encapsulated protein was performed using an IX51 Epifluorescence Microscope (Olympus Co., Japan) equipped with a mercury lamp light source and a FITC filter. For assays, the formed coacervates after incubation were centrifuged at 7,500 x g for 10 min to pellet the coacervate. The supernatant from each sample was carefully pipetted into a 96 well plate along with a serial dilution of each FITC-BSA solution, which served to generate a standard curve. The absorbance at 495 nm was measured on a Biotek Synergy H1 multimode plate reader.
CHAPTER IV

N-SUBSTITUTED DIOL MONOMER SYNTHESIS

4.1 Introduction

All diol monomers used for the generation of thermoresponsive poly(ester)s (TR-PEs) were based on N-substituted diethanolamine (DEA). In a general reaction, a commercially available or synthesized ester bearing a desired functional group was heated overnight in excess DEA to promote transamidation. The transamidation reaction yield was seen to improve under reduced pressure as to remove displaced alcohol and drive the reaction forward. The crude mixture was then purified using silica gel flash chromatography. Detailed synthetic procedures are outlined below.

4.2 Synthesis of N,N-bis(hydroxyethyl) Alkyl & Alkoxy Monomers (HEA monomers)

Scheme 4.1: Synthetic route for preparation of HEA monomers.

\[ R_1 = \text{CH}_3 \text{ or CH}_2\text{CH}_3 \]

iPr: \( R_2 = \text{n-propyl} \)
nPr: \( R_2 = \text{i-propyl} \)
Me: \( R_2 = \text{CH}_3 \)
MoMe: \( R_2 = \text{CH}_2\text{OCH}_3 \)
MoEt: \( R_2 = \text{CH}_2\text{CH}_2\text{OCH}_3 \)
The synthetic procedures used to synthesize the N-substituted diol monomers are described in this section using a modified version of the previous literature procedure. Synthesis of tert-butyl (6-(bis(2-hydroxyethyl)amino)-6-oxohexyl)carbamate (mLys), tert-butyl 4-(bis(2-hydroxyethyl)amino)-4-oxobutanoate (mGlu), N<sup>1</sup>,N<sup>1</sup>-bis(2-hydroxyethyl)-N<sup>4</sup>-prop-2-yn-1-yl)succinamide (propargyl), and N,N-bis(2-hydroxyethyl)propionamide (mAla) was undertaken according to previous literature procedures.

4.2.1 Synthesis of N,N-bis(2-hydroxyethyl)butyramide (nPr)

In a round bottom flask equipped with a magnetic stir bar was added methyl butyrate (5.00 mL, 43.9 mmol, 1 eq.) and DEA (9.23 g, 87.8 mmol, 2 eq.) which was heated at 80 °C overnight under reduced pressure. The crude compound was analyzed via TLC (10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>, ninhydrin). The product was observed at R<sub>f</sub> ~ 0.45 while unreacted DEA remained at R<sub>f</sub> ~ 0. The crude mixture was purified via silica gel flash chromatography (10-20% MeOH in CH<sub>2</sub>Cl<sub>2</sub>). The product was dried under reduced pressure to afford the pure monomer as a pale oil (5.25 g, 30.0 mmol, 65%). The monomer was characterized via NMR. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 0.93 (t, J = 7.4 Hz, 3H), 1.63 (sextet, J = 7.5 Hz, 2H), 2.35 (t, J = 7.6 Hz, 2H), 3.49 (dt, J = 11.1, 5.4 Hz, 4H), 3.76 (dt, J = 11.4, 5.5 Hz, 4H), 4.26 (br s, 2H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 13.95, 18.74, 35.48, 50.53, 52.23, 60.68, 61.16, 175.47.

4.2.2 Synthesis of N,N-bis(2-hydroxyethyl)isobutyramide (iPr)

In a round bottom flask equipped with a magnetic stir bar was added methyl isobutyrate (5.00 mL, 43.6 mmol, 1 eq.) and DEA (9.17 g, 87.2 mmol, 2 eq.) which was heated at 80 °C overnight under reduced pressure. The reduced crude compound was analyzed via
TLC (10% MeOH in CH$_2$Cl$_2$, ninhydrin). The product was observed at $R_f \approx 0.45$ while unreacted DEA remained at $R_f \approx 0$. The crude mixture was purified via silica gel flash chromatography (10-20% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford the pure monomer as a pale oil (5.27 g, 30.1 mmol, 69%). The monomer was characterized via NMR. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 1.09 (d, $J = 6.7$ Hz, 6H), 2.87 (dt, $J = 13.4$, 6.7 Hz, 1H), 3.50 (td, $J = 5.1$, 2.5 Hz, 4H), 3.76 (dt, $J = 9.9$, 5.0 Hz, 4H), 4.26 (br s, 2H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 19.64, 30.54, 50.80, 52.06, 61.07, 61.49, 179.83.

4.2.3 Synthesis of N,N-bis(2-hydroxyethyl)acetamide (Me)

In a round bottom flask equipped with a magnetic stir bar was added EtOAc (9.8 mL, 90.7 mmol, 2 eq.) and DEA (4.77 g, 45.3 mmol, 1 eq.) which was heated at 80 °C overnight under reduced pressure. The crude compound was analyzed via TLC (10% MeOH in CH$_2$Cl$_2$, ninhydrin). The product was observed at $R_f \sim 0.34$ while unreacted DEA remained at $R_f \sim 0$. The crude mixture was purified via silica gel flash chromatography (10-20% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford the pure monomer as a pale oil (4.10 g, 27.9 mmol, 61.5%). The monomer was characterized via NMR. $^1$H NMR (300 MHz; CDCl$_3$): $\delta$ 2.16 (s, 3H), 3.57-3.48 (m, 6H), 3.87-3.78 (m, 4H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 21.84, 50.04, 52.87, 60.20, 60.56, 172.91.

4.2.4 Synthesis of N,N-bis(2-hydroxyethyl)-2-methoxyacetamide (MoMe)

In a round bottom flask equipped with a magnetic stir bar was added methyl methoxyacetate (2.5 mL, 25.2 mmol, 1 eq.) and DEA (4.5 g, 42.8 mmol, 2 eq.) which was heated at 80 °C overnight. After removing displaced MeOH under reduced pressure, the crude compound was analyzed via TLC (10% MeOH in CH$_2$Cl$_2$, ninhydrin). The product was
observed at $R_f \sim 0.29$ while unreacted DEA remained at $R_f \sim 0$. The crude mixture was purified via silica gel flash chromatography (10-20% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford the pure monomer as a pale oil (3.61 g, 20.3 mmol, 80.7%). The monomer was characterized via NMR. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 3.44 (s, 3H), 3.47 (t, $J = 5.1$ Hz, 2H), 3.56 (t, $J = 5.0$ Hz, 2H), 3.78 (t, $J = 5.1$ Hz, 2H), 3.87 (t, $J = 5.0$ Hz, 2H), 4.19 (s, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 49.92, 50.86, 59.14, 60.01, 60.36, 71.01, 171.06.

4.2.5 Synthesis of $N,N$-bis(2-hydroxyethyl)-3-methoxypropanamide (MoEt)

In a round bottom flask equipped with a magnetic stir bar was added ethyl methoxacetate (3.0 mL, 25.6 mmol, 1 eq.) and DEA (6.22 g, 51.2 mmol, 2 eq.) which was heated at 80 °C overnight under reduced pressure. The crude compound was analyzed via TLC (10% MeOH in CH$_2$Cl$_2$, ninhydrin). The product was observed at $R_f \sim 0.3$ while unreacted DEA remained at $R_f \sim 0$. The crude mixture was purified via silica gel flash chromatography (5-13% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford the pure monomer as a pale oil (4.53 g, 23.7 mmol, 92.6%). The monomer was characterized via NMR. $^1$H NMR (300 MHz; CDCl$_3$): $\delta$ 2.67 (t, $J = 6.2$ Hz, 2H), 3.34 (s, 3H), 3.54 (q, $J = 5.0$ Hz, 4H), 3.70 (t, $J = 6.2$ Hz, 2H), 3.78 (dt, $J = 16.8, 5.1$ Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 33.65, 50.43, 52.17, 58.81, 60.54, 60.84, 68.82, 172.96.

4.2.6 Synthesis of 4-(bis(2-hydroxyethyl)amino)-4-oxobutyl 2-(4-isobutylphenyl)propanoate (ibuDEA)

In a round bottom flask equipped with a magnetic stir bar, $\gamma$-butyrolactone (0.7 mL, 9.2 mmol, 1 eq.), Et$_3$N (6.16 mL, 44.2 mmol, 1.02 eq.) were dissolved in MeOH (45 mL).
The reaction was allowed to stir in a microwave reactor for 2 h (Power: 100, Temp: 60ºC, PowerMax: ON, Cooling: ON). The reaction was allowed to cool and concentrated under reduced pressure. Residual MeOH was removed azeotropically with hexane under reduced pressure. The concentrated pale oil was determined to be 75% pure methyl 4-hydroxybutanoate via \(^1\)H NMR (0.98 g recovered) and used without further purification. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.91-1.82 (m, 3H), 2.30-2.22 (m, 0.67H, lactone impurity), 2.50-2.40 (m, 3H, some lactone impurity), 3.69-3.64 (m, 5H), 4.35-4.30 (m, 0.66H, lactone impurity).

In a round bottom flask equipped with a magnetic stir bar was added methyl 4-hydroxybutanoate (0.7 g, 6.0 mmol, 1 eq.), ibuprofen (1.24 g, 6.0 mmol, 1 eq.) and DPTS (1.76 g, 6.03 mmol, 1 eq.) which were dissolved in dry CH\(_2\)Cl\(_2\) (25 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N\(_2\) for 15 min. DIC was added dropwise via syringe and allowed to stir at room-temperature for 16 h. The reaction mixture was diluted with CH\(_2\)Cl\(_2\) and washed with H\(_2\)O (3 x 30 mL). The organic layer was dried over MgSO\(_4\), filtered, and concentrated under reduced pressure. The crude compound was analyzed via TLC (3% MeOH in CH\(_2\)Cl\(_2\), UV). The product was observed at \(R_f\) ~ 0.54-0.75 while two smaller impurities were observed at \(R_f\) ~ 0, 0.19. The crude mixture was purified via silica gel flash chromatography (3% MeOH in CH\(_2\)Cl\(_2\)). The product was dried under reduced pressure to afford pure methyl 4-((2-(4-isobutylphenyl)propanoyloxy)butanoate product as a pale oil (1.77 g, 1.68 mmol, 96%). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 0.89 (d, \(J = 6.6\) Hz, 6H), 1.48 (d, \(J = 7.2\) Hz, 3H), 1.91-1.84 (m, 3H), 2.27 (s, 2H some lactone impurity), 2.44 (d, \(J = 7.2\) Hz, 2H, some lactone impurity), 3.65 (s, 4H), 4.09 (s, 2H), 4.35 (s, 0.18H, lactone impurity), 7.09 (d, \(J = 8.1\) Hz, 2H), 7.18 (d, \(J = 8.2\) Hz, 2H).
In a round bottom flask equipped with a magnetic stir bar was added methyl 4-((2-(4-isobutylphenyl)propanoyloxy)butanoate (1.7 g, 6.0 mmol, 1 eq.) and DEA (1.26 g, 12.0 mmol, 2 eq.). The reaction was allowed to stir in a microwave reactor for 30 min (Power: 100, Temp: 80 ºC (15 min), 70 ºC (15 min), PowerMax: ON, Cooling: ON). The crude compound was analyzed via TLC (15% MeOH in CH₂Cl₂, UV). The product was observed at R_f ~ 0.54 that also stained under ninhydrin. The starting material was observed at R_f ~ 0.80. The crude mixture was purified via silica gel flash chromatography (15-20% MeOH in CH₂Cl₂). The product was dried under reduced pressure to afford pure product as a pale oil (1.08 g, 3.0 mmol, 50%). The monomer was characterized via NMR. ¹H NMR (300 MHz, CDCl₃): δ 0.89 (d, J = 6.6 Hz, 6H), 1.46 (t, J = 8.5 Hz, 3H), 1.89 (d, J = 29.4 Hz, 3H), 2.32 (s, 1H), 2.44 (d, J = 7.1 Hz, 2H), 2.95-2.84 (m, 2H), 3.34 (s, 1H), 3.52 (s, 2H), 3.62-3.58 (m, 1H), 3.67 (s, 2H), 3.84 (s, 2H), 4.13 (s, 1H), 7.14 (d, J = 24.2 Hz, 4H).

4.3 Synthesis of Succinamide Esters

Ethoxy succinamides were synthesized according to modified literature procedures.⁹,²⁰³

![Scheme 4.2: Synthetic route for preparation of succinamide ester intermediates.](image)

4.3.1 Synthesis of ethyl 4-(cyclopropylamino)-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, cyclopropyl amine (3.0 mL, 43.3 mmol, 1 eq.) and Et₃N (6.16 mL, 44.2 mmol, 1.02 eq.) were dissolved in dry CH₂Cl₂ (40 mL) with magnetic stirring. The reaction was cooled to 0 ºC and purged with
N₂ for 15 min. Ethyl succinyl chloride (6.16 mL, 43.3 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N₂. The solution was then added to DI water and extracted (3 x 40 mL CH₂Cl₂). The product was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the pure product as a white solid (7.33 g, 39.6 mmol, 91.2%). The product was characterized via NMR. ¹H NMR (300 MHz, CDCl₃): δ 0.46-0.41 (m, 2H), 0.71-0.65 (m, 2H), 1.20 (t, J = 7.1 Hz, 3H), 2.37 (t, J = 6.9 Hz, 2H), 2.74-2.57 (m, 3H), 4.08 (q, J = 7.1 Hz, 3H), 6.19 (s, 1H); ¹³C NMR (126 MHz, CDCl₃): δ 6.49, 14.21, 22.67, 29.65, 30.90, 60.70, 173.10.

4.3.2 Synthesis of ethyl 4-oxo-4-(pyrrolidin-1-yl)butanoate

In a round bottom flask equipped with a magnetic stir bar, pyrrolidine (3.0 mL, 36.3 mmol, 1 eq.) and Et₃N (5.16 mL, 37.0 mmol, 1.02 eq.) were dissolved in dry CH₂Cl₂ (40 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N₂ for 15 min. Ethyl succinyl chloride (5.16 mL, 36.3 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N₂. The solution was then added to DI water and extracted (3 x 40 mL CH₂Cl₂). The product was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the pure product as a pale oil (6.93 g, 34.8 mmol, 96%). The product was characterized via NMR. ¹H NMR (300 MHz, CDCl₃): δ 1.15 (t, J = 7.1 Hz, 3H), 1.91-1.70 (m, 4H), 2.58-2.43 (m, 4H), 3.35 (t, J = 6.8 Hz, 4H), 4.03 (q, J = 7.1 Hz, 2H); ¹³C NMR (126 MHz, CDCl₃): δ 14.13, 24.33, 26.00, 29.09, 45.62, 46.37, 60.41, 169.65, 173.17.
4.3.3 Synthesis of ethyl 4-(diethylamino)-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, diethylamine (3.0 mL, 29.0 mmol, 1 eq.) and Et₃N (4.13 mL, 29.6 mmol, 1.02 eq.) were dissolved in dry CH₂Cl₂ (40 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N₂ for 15 min. Ethyl succinyl chloride (4.13 mL, 29.0 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N₂. The solution was then added to DI water and extracted (3 x 40 mL CH₂Cl₂). The product was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the pure product as a yellow oil (5.55 g, 27.6 mmol, 96%). The product was characterized via NMR.¹HNMR (300 MHz, CDCl₃): δ 1.08 (t, J = 7.1 Hz, 3H), 1.21 (dt, J = 17.7, 7.1 Hz, 6H), 2.68-2.56 (m, 4H), 3.39-3.28 (m, J = 11.3, 7.1 Hz, 4H), 4.12 (q, J = 7.1 Hz, 2H); ¹³CNMR (126 MHz, CDCl₃): δ 13.13, 14.24, 27.93, 29.56, 40.34, 41.86, 60.49, 76.91, 77.16, 77.41, 170.30, 173.29.

4.3.4 Synthesis of ethyl 4-(isopropylamino)-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, isopropylamine (3.0 mL, 35.1 mmol, 1 eq.) and Et₃N (4.99 mL, 35.8 mmol, 1.02 eq.) were dissolved in dry CH₂Cl₂ (40 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N₂ for 15 min. Ethyl succinyl chloride (4.99 mL, 35.8 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N₂. The solution was then added to DI water and extracted (3 x 40 mL CH₂Cl₂). The product was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the pure product as a white crystalline solid (6.17
g, 32.9 mmol, 93.9%). The product was characterized via NMR. $^1$H NMR (300 MHz, CDCl$_3$): δ 1.06 (d, $J = 6.6$ Hz, 6H), 1.18 (t, 3H), 2.36 (t, $J = 7.0$ Hz, 2H), 2.57 (t, $J = 6.8$ Hz, 2H), 4.10-3.95 (m, 3H), 5.82 (br s, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$): δ 14.24, 22.76, 29.81, 31.32, 41.45, 60.68, 170.56, 173.11.

4.3.5 Synthesis of ethyl 4-oxo-4-(propylamino)butanoate

In a round bottom flask equipped with a magnetic stir bar, propylamine (3.0 mL, 36.5 mmol, 1 eq.) and Et$_3$N (5.19 mL, 37.2 mmol, 1.02 eq.) were dissolved in dry CH$_2$Cl$_2$ (40 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N$_2$ for 15 min. Ethyl succinyl chloride (5.19 mL, 36.5 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N$_2$. The solution was then added to DI water and extracted (3 x 40 mL CH$_2$Cl$_2$). The product was dried over MgSO$_4$, filtered, and concentrated under reduced pressure to afford the pure product as a pale oil (6.72 g, 35.9 mmol, 98.4%). The product was characterized via NMR. $^1$H NMR (300 MHz, CDCl$_3$): δ 0.89 (t, $J = 7.4$ Hz, 3H), 1.23 (t, $J = 7.1$ Hz, 3H), 1.49 (sextet, $J = 7.3$ Hz, 2H), 2.44 (t, $J = 6.8$ Hz, 2H), 2.64 (t, $J = 6.8$ Hz, 2H), 3.19 (q, $J = 7.1$ Hz, 2H), 4.12 (q, $J = 7.1$ Hz, 2H), 5.79 (s, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$): δ 11.35, 14.20, 22.86, 29.79, 31.14, 41.34, 60.66, 171.45, 173.11.

4.3.6 Synthesis of ethyl 4-(bis(2-methoxyethyl)amino)-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, bis(2-methoxyethyl)amine (3.0 mL, 20.3 mmol, 1 eq.) and Et$_3$N (2.89 mL, 20.7 mmol, 1.02 eq.) were dissolved in dry CH$_2$Cl$_2$ (30 mL) with magnetic stirring. The reaction was cooled to 0 °C
and purged with N₂ for 15 min. Ethyl succinyl chloride (2.89 mL, 20.3 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N₂. The solution was then added to DI water and extracted (3 x 40 mL CH₂Cl₂). The product was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the pure product as a pale oil (4.01 g, 17.4 mmol, 85.7%). The product was characterized via NMR. ¹H NMR (300 MHz; CDCl₃): δ 1.25 (t, J = 7.1 Hz, 3H), 2.73-2.60 (m, 4H), 3.32 (d, J = 6.9 Hz, 6H), 3.57-3.50 (m, 9H), 4.13 (q, J = 7.1 Hz, 2H). ¹³C NMR (126 MHz; CDCl₃): δ 14.13, 28.00, 29.45, 46.43, 48.65, 58.69, 58.98, 60.35, 70.66, 71.10, 171.70, 173.14

4.3.7 Synthesis of ethyl 4-morpholino-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, morpholine (3.0 mL, 34.7 mmol, 1 eq.) and Et₃N (4.9 mL, 35.4 mmol, 1.02 eq.) were dissolved in dry CH₂Cl₂ (40 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N₂ for 15 min. Ethyl succinyl chloride (4.9 mL, 34.7 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N₂. The solution was then added to DI water and extracted (3 x 40 mL CH₂Cl₂). The product was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the pure product as an off-white solid (6.82 g, 31.7 mmol, 91.3%). The product was characterized via NMR. ¹H NMR (300 MHz, CDCl₃): δ 1.29-1.24 (m, 3H), 2.70-2.58 (m, 4H), 3.49 (t, J = 4.7 Hz, 2H), 3.63 (s, 2H), 3.67 (t, J = 4.5 Hz, 4H), 4.15 (q, J = 7.1 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃): δ 14.17, 27.68, 29.22, 42.07, 45.72, 60.53, 66.51, 66.82, 169.91, 172.97.
4.3.8 Synthesis of ethyl 4-((2-methoxyethyl)amino)-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, 2-methoxyethylamine (1.83 mL, 21.1 mmol, 1 eq.) and Et<sub>3</sub>N (3.00 mL, 21.5 mmol, 1.02 eq.) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (30 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N<sub>2</sub> for 15 min. Ethyl succinyl chloride (3.00 mL, 21.1 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N<sub>2</sub>. The solution was then added to DI water and extracted (3 x 40 mL CH<sub>2</sub>Cl<sub>2</sub>). The product was dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure to afford the pure product as a pale oil (4.02 g, 19.9 mmol, 94.2%). The product was characterized via NMR. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): δ 1.25 (t, J = 7.1 Hz, 3H), 2.47 (t, J = 7.0 Hz, 2H), 2.64 (q, J = 6.8 Hz, 2H), 3.35 (s, 3H), 3.44-3.44 (m, 4H), 4.14 (q, J = 7.1 Hz, 2H), 5.96 (s, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 14.14, 29.58, 30.89, 39.22, 58.64, 60.57, 71.15, 171.51, 172.89.

4.3.9 Synthesis of ethyl 4-((2-ethoxyethyl)amino)-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, 2-ethoxyethylamine (2.20 mL, 21.1 mmol, 1 eq.) and Et<sub>3</sub>N (3.00 mL, 21.5 mmol, 1.02 eq.) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (30 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N<sub>2</sub> for 15 min. Ethyl succinyl chloride (3.00 mL, 21.8 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N<sub>2</sub>. The solution was then added to DI water and washed (3 x 40 mL DI water). The product was dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure to afford the pure product as a pale oil.
The product was characterized via NMR. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 1.23 (dt, \(J = 25.0, 7.1\) Hz, 6H), 2.48 (t, \(J = 6.9\) Hz, 2H), 2.66 (t, \(J = 6.9\) Hz, 2H), 3.52-3.42 (m, 6H), 4.14 (q, \(J = 7.1\) Hz, 2H), 5.97 (s, 1H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta\) 14.33, 15.26, 29.75, 31.19, 39.54, 60.79, 66.57, 69.14, 171.49, 173.06.

4.3.10 Synthesis of ethyl 4-oxo-4-(((tetrahydrofuran-2-yl)methyl)amino)butanoate

In a round bottom flask equipped with a magnetic stir bar, tetrahydrofurfurylamine (2.18 mL, 21.1 mmol, 1 eq.) and Et\(_3\)N (3.00 mL, 21.5 mmol, 1.02 eq.) were dissolved in dry CH\(_2\)Cl\(_2\) (30 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N\(_2\) for 15 min. Ethyl succinyl chloride (3.00 mL, 21.8 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N\(_2\). The solution was then added to DI water and extracted (3 x 40 mL CH\(_2\)Cl\(_2\)). The product was dried over MgSO\(_4\), filtered, and concentrated under reduced pressure to afford the pure product as a pale oil (4.44 g, 19.4 mmol, 91.7%). The product was characterized via NMR. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.25 (t, \(J = 7.1\) Hz, 3H), 1.58-1.50 (m, 1H), 2.01-1.84 (m, 3H), 2.48 (t, \(J = 6.7\) Hz, 2H), 2.68-2.61 (m, 2H), 3.14 (ddd, \(J = 13.8, 7.4, 4.9\) Hz, 1H), 3.57 (ddd, \(J = 13.8, 6.5, 3.4\) Hz, 1H), 3.78-3.70 (m, 1H), 3.87-3.82 (m, 1H), 3.97-3.89 (m, 1H), 4.14 (q, \(J = 7.1\) Hz, 2H), 5.94 (s, 1H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 14.10, 25.74, 28.54, 29.55, 30.83, 43.17, 60.49, 67.95, 77.70, 171.49, 172.82.

4.3.11 Synthesis of ethyl 4-oxo-4-(piperidin-1-yl)butanoate

In a round bottom flask equipped with a magnetic stir bar, piperidine (2.08 mL, 21.1 mmol, 1 eq.) and Et\(_3\)N (3.00 mL, 21.5 mmol, 1.02 eq.) were dissolved in dry CH\(_2\)Cl\(_2\)
(30 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N₂ for 15 min. Ethyl succinyl chloride (3.00 mL, 21.1 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N₂. The solution was then added to DI water and extracted (3 x 40 mL CH₂Cl₂). The product was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the pure product as a yellow oil (4.13 g, 19.4 mmol, 91.8%). The product was characterized via NMR. ¹H NMR (300 MHz, CDCl₃): δ 1.25 (d, J = 14.3 Hz, 3H), 1.58 (dq, J = 18.7, 5.7 Hz, 6H), 2.63 (dd, J = 4.5, 3.4 Hz, 4H), 3.41 (t, J = 5.3 Hz, 2H), 3.54 (t, J = 5.5 Hz, 2H), 4.14 (q, J = 7.1 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃): δ 13.93, 24.29, 25.29, 26.11, 27.66, 29.20, 42.56, 46.10, 60.10, 169.07, 172.87.

4.3.12 Synthesis of ethyl 4-(dibutylamino)-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, di-n-butylamine (3.0 mL, 17.8 mmol, 1 eq.) and Et₃N (2.53 mL, 18.2 mmol, 1.02 eq.) were dissolved in dry CH₂Cl₂ (30 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N₂ for 15 min. Ethyl succinyl chloride (3.0 mL, 17.8 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N₂. The solution was then added to DI water and extracted (3 x 40 mL CH₂Cl₂). The product was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the pure product as a pale yellow oil (4.42 g, 17.2 mmol, 96.5%). The product was characterized via NMR. ¹H NMR (300 MHz, CDCl₃): δ 0.93 (dt, J = 13.2, 7.3 Hz, 6H), 1.37-1.23 (m, 8H), 1.62-1.44 (m, 4H), 2.69-2.57 (m, 4H), 3.27 (dt, J = 18.4, 7.7 Hz, 4H), 4.14 (q, J = 7.1 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃): δ
4.3.13 Synthesis of ethyl 4-(heptylamino)-4-oxobutanoate

In a round bottom flask equipped with a magnetic stir bar, heptylamine (3.0 mL, 20.2 mmol, 1 eq.) and Et\textsubscript{3}N (2.87 mL, 20.6 mmol, 1.02 eq.) were dissolved in dry CH\textsubscript{2}Cl\textsubscript{2} (30 mL) with magnetic stirring. The reaction was cooled to 0 °C and purged with N\textsubscript{2} for 15 min. Ethyl succinyl chloride (2.87 mL, 20.2 mmol, 1 eq.) was added dropwise via syringe and the reaction became an opaque white solution. The reaction was brought to room-temperature and allowed to stir for 1 h under N\textsubscript{2}. The solution was then added to DI water and extracted (3 x 40 mL CH\textsubscript{2}Cl\textsubscript{2}). The product was dried over MgSO\textsubscript{4}, filtered, and concentrated under reduced pressure to afford the pure product as a pale yellow oil (4.42 g, 17.2 mmol, 96.5%). The product was characterized via NMR. \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta \) 0.87 (t, \(J = 6.8 \) Hz, 3H), 1.28-1.23 (m, 11H), 1.48 (t, \(J = 6.3 \) Hz, 2H), 2.44 (t, \(J = 6.8 \) Hz, 2H), 2.64 (q, \(J = 6.6 \) Hz, 2H), 3.23 (td, \(J = 7.1, 5.9 \) Hz, 2H), 4.14 (q, \(J = 7.1 \) Hz, 2H), 5.63 (s, 1H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): \(\delta \) 13.98, 14.12, 22.53, 26.84, 28.93, 29.56, 29.72, 31.02, 31.71, 39.61, 60.55, 171.36, 173.02.

4.4 Synthesis of \(N,N\)-bis(hydroxyethyl) Succinamide Monomers (HESA monomers)

The synthetic procedures used to synthesize the \(N\)-substituted diol monomers are described in this section using a modified version of the previous literature procedure.\textsuperscript{9}
Scheme 4.3: Synthetic route for preparation of HESA monomers.

4.4.1 Synthesis of \(N^1\)-cyclopropyl-\(N^4\),\(N^4\)-bis(2-hydroxyethyl)succinamide (cPrA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-(cyclopropylamino)-4-oxobutanoate (5.17 g, 27.9 mmol, 1 eq.) and DEA (5.87 g, 55.9 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide (\(R_f \sim 0.56\)) was observed along with the desired compound (\(R_f \sim 0.40\)). The crude mixture was purified via silica gel flash chromatography (10-15% MeOH in CH\(_2\)Cl\(_2\)). The product was dried under reduced pressure to afford pure monomer as a pale solid (5.64 g, 23.1 mmol, 82.6%). The product was characterized via NMR.

\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta 0.50-0.44\) (m, 2H), 0.75-0.68 (m, 2H), 2.48 (t, \(J = 6.4\) Hz, 2H), 2.61-2.68 (m, 1H), 2.73 (t, \(J = 6.5\) Hz, 2H), 3.53 (q, \(J = 5.0\) Hz, 5H), 3.79 (br s, 5H), 4.03 (br s, 1H), 4.47 (br s, 1H), 6.45 (br s, 1H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta 6.29, 22.63, 28.67, 31.20, 50.48, 52.13, 60.43, 173.92, 174.50\).
4.4.2 Synthesis of \(N,N\)-bis(2-hydroxyethyl)-4-oxo-4-(pyrrolidin-1-yl)butanamide (PyrA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-oxo-4-(pyrrolidin-1-yl)butanoate (5.98 g, 30.0 mmol, 1 eq.) and DEA (6.30 g, 60.0 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide (\(R_f \sim 0.56\)) was observed along with the desired compound (\(R_f \sim 0.40\)). The crude mixture was purified via silica gel flash chromatography (10-20% MeOH in \(CH_2Cl_2\)). The product was dried under reduced pressure to afford pure monomer as a pale solid (4.90 g, 19.0 mmol, 63%). The product was characterized via NMR. \(^1H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.98-1.77 (m, 4H), 2.67 (q, \(J = 6.0\) Hz, 4H), 3.41 (dt, \(J = 10.5, 6.8\) Hz, 4H), 3.54 (dt, \(J = 15.4, 4.9\) Hz, 4H), 3.78 (dd, \(J = 9.7, 4.7\) Hz, 4H), 4.03 (br s, 1H), 4.78 (br s, 1H); \(^13C\) NMR (126 MHz, CDCl\(_3\)): \(\delta\) 24.27, 25.89, 27.71, 29.91, 45.74, 46.51, 50.59, 52.30, 60.75, 170.87, 173.86.

4.4.3 Synthesis of \(N^1,N^1\)-diethyl-\(N^4,N^4\)-bis(2-hydroxyethyl)succinamide (dEtA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-(diethylamino)-4-oxobutanoate (6.00 g, 29.8 mmol, 1 eq.) and DEA (6.27 g, 59.6 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide (\(R_f \sim 0.53\)) was observed along with the desired compound (\(R_f \sim 0.39\)). The crude mixture was purified via silica gel flash chromatography (15% MeOH in \(CH_2Cl_2\)). The product was dried under reduced pressure to afford pure monomer as a pale
oil (3.41 g, 13.1 mmol, 44%). The product was characterized via NMR. $^1$H NMR (300 MHz, CDCl$_3$): δ0.93 (t, 3H), δ1.62 (m, 2H), δ2.34 (t, 2H), δ3.47 (m, 4H), δ3.74 (m, 4H), δ4.23 (br, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$): δ 12.97, 13.98, 28.05, 28.55, 40.47, 42.04, 50.67, 52.38, 60.82, 171.51, 174.05.

4.4.4 Synthesis of $N^1,N^1$-bis(2-hydroxyethyl)-$N^4$-isopropylsuccinamide (iPrA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-(isopropylamino)-4-oxobutanoate (6.34 g, 33.9 mmol, 1 eq.) and DEA (7.12 g, 67.7 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide ($R_f \sim 0.52$) was observed along with the desired compound ($R_f \sim 0.40$). The crude mixture was purified via silica gel flash chromatography (10-15% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford pure monomer as a pale solid (4.59 g, 18.6 mmol, 55%). The product was characterized via NMR. $^1$H NMR (300 MHz, CDCl$_3$): δ 1.04 (d, $J = 6.5$ Hz, 6H), 2.40 (t, $J = 6.7$ Hz, 2H), 2.64 (t, $J = 6.7$ Hz, 2H), 3.46 (dt, $J = 9.3$, 4.8 Hz, 4H), 3.69 (s, 5H), 3.90 (dq, $J = 13.8$, 6.8 Hz, 1H), 4.54 (br s, 1H), 4.89 (br s, 1H), 6.49 (d, $J = 7.8$ Hz, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$): δ 22.65, 28.73, 31.57, 41.58, 50.62, 52.24, 60.73, 171.93, 173.97.

4.4.5 Synthesis of $N^1,N^1$-bis(2-hydroxyethyl)-$N^4$-propylsuccinamide (nPrA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-oxo-4-(propylamino)butanoate (6.50 g, 34.7 mmol, 1 eq.) and DEA (7.30 g, 69.5 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount
of unreacted ethoxy amide ($R_f \sim 0.55$) was observed along with the desired compound ($R_f \sim 0.46$). The crude mixture was purified via silica gel flash chromatography (15% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford pure monomer as a light orange solid (3.21 g, 13.0 mmol, 38%). The product was characterized via NMR. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.07 (t, $J = 7.1$ Hz, 3H), 1.20 (t, $J = 7.2$ Hz, 3H), 2.74-2.71 (m, 4H), 3.29-3.37 (m, 4H), 3.56 (dt, $J = 14.9$, 4.4 Hz, 4H), 3.78-3.83 (m, 4H), 4.65 (br s, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 11.47, 22.85, 28.66, 31.63, 41.53, 50.96, 52.53, 60.85, 61.23, 172.84, 174.31.

4.4.6 Synthesis of $N^1, N^1$-bis(2-hydroxyethyl)-$N^4, N^4$-bis(2-methoxyethyl)succinamide (bMoEtA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-(bis(2-methoxyethyl)amino)-4-oxobutanoate (3.89 g, 16.6 mmol, 1 eq.) and DEA (3.49 g, 33.2 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide ($R_f \sim 0.56$) was observed along with the desired compound ($R_f \sim 0.40$). The crude mixture was purified via silica gel flash chromatography (10-20% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford pure monomer as a pale oil (1.99 g, 6.80 mmol, 41%). The product was characterized via NMR. $^1$H NMR (300 MHz; CDCl$_3$): $\delta$ 2.71-2.67 (m, 2H), 2.87-2.83 (m, 2H), 3.32 (d, $J = 10.1$ Hz, 6H), 3.61-3.44 (m, 12H), 3.83 (dt, $J = 9.0$, 4.6 Hz, 4H); $^{13}$C NMR (126 MHz; CDCl$_3$): $\delta$ 14.13, 28.00, 29.45, 46.43, 48.65, 58.69, 58.98, 60.35, 70.66, 71.10, 171.70, 173.14.
4.4.7 Synthesis of N,N-bis(2-hydroxyethyl)-4-morpholino-4-oxobutanamide (MorA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-morpholino-4-oxobutanoate (6.26 g, 29.1 mmol, 1 eq.) and DEA (6.11 g, 58.2 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide (R_f ~ 0.53) was observed along with the desired compound (R_f ~ 0.42). The crude mixture was purified via silica gel flash chromatography (10-20% MeOH in CH₂Cl₂). The product was dried under reduced pressure to afford pure monomer as a pale white solid (6.79 g, 25.0 mmol, 86.1%). The product was characterized via NMR. 

$^1$H NMR (500 MHz, CDCl₃): δ 2.73 (s, 4H), 3.50 (t, J = 4.8 Hz, 2H), 3.57 (dq, J = 15.3, 5.1 Hz, 6H), 3.66 (dt, J = 18.2, 4.7 Hz, 4H), 3.82 (dt, J = 13.6, 4.8 Hz, 4H), 4.27 (s, 1H); $^{13}$C NMR (126 MHz, CDCl₃): δ 27.84, 28.85, 42.35, 45.94, 50.85, 52.60, 61.16, 61.44, 66.59, 66.91, 171.25, 174.43.

4.4.8 Synthesis of N¹,N¹-bis(2-hydroxyethyl)-N⁴-(2-methoxyethyl)succinamide (MoEtA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-((2-methoxyethyl)amino)-4-oxobutanoate (3.21 g, 15.8 mmol, 1 eq.) and DEA (3.32 g, 31.6 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (10% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide (R_f ~ 0.46) was observed along with the desired compound (R_f ~ 0.25). The crude mixture was purified via silica gel flash chromatography (10-
15% MeOH in CH₂Cl₂). The product was dried under reduced pressure to afford pure monomer as a pale oil (2.89 g, 11.0 mmol, 69.8%). The product was characterized via NMR. **¹H NMR (500 MHz; CDCl₃):** δ 2.61 (t, J = 6.3 Hz, 2H), 2.74 (dd, J = 7.4, 5.4 Hz, 2H), 3.36 (s, 3H), 3.42 (dt, J = 10.4, 5.3 Hz, 4H), 3.56 (s, 4H), 3.70 (s, 1H), 3.83 (t, J = 4.6 Hz, 4H), 4.19 (s, 1H), 6.28 (s, 1H); **¹³C NMR (126 MHz, CDCl₃):** δ 28.51, 31.57, 39.37, 50.97, 52.57, 58.81, 61.02, 61.31, 71.23, 172.85, 174.24.

4.4.9 Synthesis of N¹-(2-ethoxyethyl)-N⁴,N⁴-bis(2-hydroxyethyl)succinamide (EoEtA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-((2-ethoxyethyl)amino)-4-oxobutanoate (3.06 g, 14.1 mmol, 1 eq.) and DEA (2.96 g, 28.2 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide (Rᶠ ~ 0.60) was observed along with the desired compound (Rᶠ ~ 0.41). The crude mixture was purified via silica gel flash chromatography (5-15% MeOH in CH₂Cl₂). The product was dried under reduced pressure to afford pure monomer as a pale oil (1.96 g, 7.10 mmol, 50.4%). The product was characterized via NMR. **¹H NMR (500 MHz, CDCl₃):** δ 1.20 (t, J = 7.0 Hz, 3H), 2.60 (t, J = 6.3 Hz, 2H), 2.73 (dd, J = 7.4, 5.3 Hz, 2H), 3.41 (quintet, J = 5.1 Hz, 2H), 3.49 (dt, J = 13.0, 6.3 Hz, 4H), 3.55 (q, J = 4.2 Hz, 4H), 3.73 (s, 1H), 3.82 (s, 4H), 4.25 (s, 1H), 6.26 (s, 1H); **¹³C NMR (126 MHz, CDCl₃):** δ 15.23, 28.43, 31.57, 39.55, 50.96, 52.59, 61.29, 66.54, 172.79, 174.25.
4.4.10 Synthesis of \( N^1,N^1\)-bis(2-hydroxyethyl)-\( N^4\)-((tetrahydrofuran-2-yl)methyl)succinamide (THFA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-oxo-4-(((tetrahydrofuran-2-yl)methyl)amino)butanoate (4.19 g, 18.3 mmol, 1 eq.) and DEA (3.85 g, 36.6 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (15% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide (\( R_f \sim 0.56 \)) was observed along with the desired compound (\( R_f \sim 0.40 \)). The crude mixture was purified via silica gel flash chromatography (5-15% MeOH in CH\(_2\)Cl\(_2\)). The product was dried under reduced pressure to afford pure monomer as a pale oil (2.55 g, 8.84 mmol, 48.3%). The product was characterized via NMR. \(^1\)H NMR (300 MHz; CDCl\(_3\)): \( \delta \) 2.71-2.67 (m, 2H), 2.87-2.83 (m, 2H), 3.32 (d, \( J = 10.1 \) Hz, 6H), 3.61-3.44 (m, 12H), 3.83 (dt, \( J = 9.0, 4.6 \) Hz, 4H); \(^13\)C NMR (75 MHz, CDCl\(_3\)): \( \delta \) 25.71, 28.64, 28.67, 31.27, 43.33, 50.42, 52.04, 60.46, 60.61, 67.99, 77.76, 172.95, 173.72.

4.4.11 Synthesis of \( N,N\)-bis(2-hydroxyethyl)-4-oxo-4-(piperidin-1-yl)butanamide (PPDA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-oxo-4-(piperidin-1-yl)butanoate (3.79 g, 17.8 mmol, 1 eq.) and DEA (3.74 g, 35.6 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (10% MeOH in DCM, ninhydrin). A small amount of unreacted ethoxy amide (\( R_f \sim 0.42 \)) was observed along with the desired compound (\( R_f \sim 0.36 \)). The crude mixture was purified via silica gel flash chromatography (0-15% MeOH
in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford pure monomer as a pale oil (2.54 g, 9.31 mmol, 52.4%). The product was characterized via NMR. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.63-1.51 (m, 6H), 2.72 (dd, $J = 16.8$, 5.5 Hz, 4H), 3.43 (d, $J = 5.1$ Hz, 2H), 3.61-3.49 (m, 6H), 3.83 (dd, $J = 14.7$, 3.6 Hz, 4H), 4.64 (s, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 24.57, 25.61, 26.37, 27.94, 29.06, 43.17, 46.62, 50.93, 52.69, 61.18, 61.30, 170.65, 174.65.

4.4.12 Synthesis of $N^1,N^1$-dibutyl-$N^4,N^4$-bis(2-hydroxyethyl)succinamide (dnBuA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-(dibutylamino)-4-oxobutanoate (4.15 g, 16.1 mmol, 1 eq.) and DEA (3.39 g, 32.3 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (10% MeOH in DCM, ninhydrin). The desired compound was observed at $R_f$ ~ 0.36. The crude mixture was purified via silica gel flash chromatography (0-12.5% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford pure monomer as a pale oil (2.49 g, 7.86 mmol, 48.8%). The product was characterized via NMR. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 0.93 (dt, $J = 25.9$, 7.3 Hz, 6H), 1.31 (dq, $J = 32.2$, 7.5 Hz, 4H), 1.47 (dt, $J = 14.9$, 7.5 Hz, 2H), 1.58 (dt, $J = 15.1$, 7.6 Hz, 2H), 2.74 (dd, $J = 19.5$, 6.0 Hz, 4H), 3.26 (dt, $J = 13.8$, 7.2 Hz, 4H), 3.58 (dt, $J = 25.2$, 4.6 Hz, 4H), 3.68 (s, 1H), 3.83 (d, $J = 19.6$ Hz, 4H), 4.61-4.57 (m, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 13.93, 14.00, 20.31, 27.99, 29.22, 29.96, 30.95, 46.14, 47.93, 51.04, 52.82, 61.24, 61.30, 172.04, 174.70.
4.4.13 Synthesis of $N^1$-heptyl-$N^4$-$N^4$-bis(2-hydroxyethyl)succinamide (HepA)

In a round bottom flask equipped with a magnetic stir bar was added ethyl 4-(heptylamine)-4-oxobutanoate (3.51 g, 14.4 mmol, 1 eq.) and DEA (3.03 g, 28.9 mmol, 2 eq.) and allowed to heat at 80 °C overnight under magnetic stirring and reduced pressure. The crude compound was analyzed via TLC (10% MeOH in DCM, ninhydrin). The desired compound was observed at $R_f \sim 0.36$. The crude mixture was purified via silica gel flash chromatography (10-20% MeOH in CH$_2$Cl$_2$). The product was dried under reduced pressure to afford pure monomer as a waxy yellow solid (0.92 g, 3.04 mmol, 21.0%). The product was characterized via NMR. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 0.87 (t, $J = 6.7$ Hz, 3H), 1.28 (s, 8H), 1.46 (t, $J = 6.5$ Hz, 2H), 2.54 (t, $J = 6.4$ Hz, 2H), 2.73 (t, $J = 6.4$ Hz, 2H), 3.19 (q, $J = 6.6$ Hz, 2H), 3.55 (t, $J = 5.0$ Hz, 4H), 3.80 (s, 5H), 4.31 (s, 1H), 6.04 (s, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 14.19, 22.72, 27.00, 28.60, 29.08, 29.64, 31.69, 31.88, 39.89, 50.99, 52.55, 60.93, 61.34, 172.69, 174.35.
CHAPTER V

POLYESTERIFICATION OF N-SUBSTITUTED DIOL MONOMERS

5.1 Introduction

Carbodiimide-mediated polyesterification was used to generate thermoresponsive poly(ester)s (TR-PEs) from N-substituted diols. This route allows for the generation of high molecular weight PEs bearing sensitive functional groups that might not be possible using other polyesterification methods. In a general reaction, an equimolar ratio of diol and diacid were taken into flask containing a small amount of 4-(dimethylamino) pyridinium 4-toluene sulfonate (DPTS) and dry CH₂Cl₂. The mixture was then cooled and N,N’-diisopropyl-carbodiimide (DIC) was added dropwise and allowed to stir at room-temperature for 1 – 4 days. Detailed synthetic procedures are described below.

5.2 General Procedure for Polyesterification of N-Substituted Diol Monomers

\[
\text{HO}_\text{N}_\text{OH} \quad \xrightarrow{\text{DIC, DPTS, CH}_2\text{Cl}_2, 48\text{ hrs, RT}} \quad \text{O}_\text{O} \quad \xrightarrow{\text{DIC, DPTS, CH}_2\text{Cl}_2, 48\text{ hrs, RT}} \quad \text{O}_\text{N}_\text{O}_\text{R} \quad \xrightarrow{\text{DIC, DPTS, CH}_2\text{Cl}_2, 48\text{ hrs, RT}} \quad \text{O}_\text{O}_\text{R} \quad \xrightarrow{\text{DIC, DPTS, CH}_2\text{Cl}_2, 48\text{ hrs, RT}} \quad \text{O}_\text{O}_\text{R}
\]

TR-iPrPE: \( R = \text{i-propyl} \)
TR-nPrPE: \( R = \text{n-propyl} \)
TR-MePE: \( R = \text{CH}_3 \)
TR-MoMePE: \( R = \text{CH}_2\text{OCH}_3 \)
TR-MoEtPE: \( R = \text{CH}_2\text{CH}_2\text{OCH}_3 \)

Polyesters were prepared according to previously established methods using room-temperature carbodiimide mediated polymerization.⁹
5.2.1 Synthesis of TR-iPrPE

In a round bottom flask equipped with a magnetic stir bar, \(N,N\)-bis(2-hydroxyethyl)isobutyramide (1.16 g, 6.63 mmol, 1 equiv.), succinic acid (783 mg, 6.63 mmol, 1 equiv.), and DPTS (775 mg, 2.65 mmol, 0.4 equiv.) were dissolved in dry \(\text{CH}_2\text{Cl}_2\) (6 mL, 1 mL / 100 mg succinic acid) and purged with \(\text{N}_2\) for 15 min with stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (3.1 mL, 19.9 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 44 h under \(\text{N}_2\). The crude reaction mixture was diluted with \(\text{CH}_2\text{Cl}_2\) and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure and purified via precipitation into cold \(\text{MeOH}\) (2 x 200 mL). The purified polymer was dried under reduced pressure to obtain the polymer as a viscous transparent pale oil. The resultant polyester was characterized by SEC, DSC, TGA and NMR. Recovered = 396 mg, \(M_n = 17.1\) kDa, \(\mathcal{D}_M = 1.37\), \(T_g = -2\) °C. 

\(^1\text{H NMR (300 MHz, CDCl}_3\):} \delta 0.96 (t, \(J = 7.4\) Hz, 3H), 1.66 (sextet, \(J = 7.4\) Hz, 2H), 2.33 (t, \(J = 7.4\) Hz, 2H), 2.62 (t, \(J = 3.2\) Hz, 4H), 3.61 (q, \(J = 5.3\) Hz, 4H), 4.24-4.22 (m, 4H). 

Urea impurity from polymerization observable at \(\delta 1.19\) and \(\delta 4.01\).

5.2.2 Synthesis of TR-nPrPE

In a round bottom flask equipped with a magnetic stir bar, \(N,N\)-bis(2-hydroxyethyl)butyramide (1.106 g, 6.31 mmol, 1 equiv.), succinic acid (745 mg, 6.31 mmol, 1 equiv.), and DPTS (736 mg, 2.52 mmol, 0.4 equiv.) were dissolved in dry \(\text{CH}_2\text{Cl}_2\) (9 mL, 1 mL / 100 mg succinic acid) and purged with \(\text{N}_2\) for 15 min with stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0
°C and DIC (3.0 mL, 18.9 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 49 h under N₂. The crude reaction mixture was diluted with CH₂Cl₂ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure and purified via precipitation into cold MeOH (2 x 200 mL). The purified polymer was dried under reduced pressure to obtain the polymer as a viscous transparent pale oil. The resultant polyester was characterized by SEC, DSC, TGA and NMR. Recovered = 756 mg, $M_n = 25.3$ kDa, $D_M = 1.57$, $T_g = -20$ °C. $^1$H NMR (300 MHz, CDCl₃): δ 1.11 (d, $J = 6.7$ Hz, 6H), 1.11 (d, $J = 6.7$ Hz, 6H), 2.60 (t, $J = 2.8$ Hz, 4H), 2.60 (t, $J = 2.8$ Hz, 4H), 2.82 (septet, $J = 13.4$, 6.7 Hz, 1H), 3.65-3.57 (m, 4H), 3.65-3.57 (m, 4H), 4.22-4.21 (m, 4H), 4.22-4.21 (m, 4H). Urea impurity from polymerization observable at δ1.19 and δ4.01

5.2.3 Synthesis of TR-MePE

In a round bottom flask equipped with a magnetic stir bar, N,N-bis(2-hydroxyethyl)acetamide (0.643 g, 4.37 mmol, 1 equiv.), succinic acid (514 mg, 4.37 mmol, 1 equiv.), and DPTS (511 mg, 1.75 mmol, 0.4 equiv.) were dissolved in dry CH₂Cl₂ (5 mL, 1 mL / 100 mg succinic acid) and purged with N₂ for 15 min with stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (2.1 mL, 13.1 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 88 h under N₂. The crude reaction mixture was diluted with CH₂Cl₂ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure and purified via precipitation into cold MeOH (2 x 200 mL). The purified polymer was dried under reduced pressure to
obtain the polymer as a viscous transparent pale oil. The resultant polyester was characterized by SEC, DSC, TGA and NMR. Recovered = 484 mg, $M_n = 45.3$ kDa, $D_M = 1.3$, $T_g = -5 \, ^\circC$. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.12 (s, 3H), 2.61 (t, $J = 3.8$ Hz, 4H), 3.60 (q, $J = 6.5$ Hz, 4H), 4.23 (t, $J = 5.4$ Hz, 4H).

5.2.4 Synthesis of TR-MoMePE

In a round bottom flask equipped with a magnetic stir bar, $N,N$-bis(2-hydroxyethyl)-2-methoxyacetamide (0.974 g, 5.49 mmol, 1 equiv.), succinic acid (649 mg, 5.49 mmol, 1 equiv.), and DPTS (642 mg, 2.20 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (12 mL, 2 mL / 1 mmol succinic acid) and purged with N$_2$ for 15 min with stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 $^\circC$ and DIC (2.6 mL, 16.5 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 80 h under N$_2$. The crude reaction mixture was diluted with CH$_2$Cl$_2$ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure and purified via precipitation into cold MeOH:iPrOH (2 x 200 mL). The purified polymer was dried under reduced pressure to obtain the polymer as a viscous transparent pale oil. The resultant polyester was characterized by SEC, DSC, TGA and NMR. Recovered = 703 mg, $M_n = 23.1$ kDa, $D_M = 1.43$, $T_g = -9 \, ^\circC$, $T_{deg} = 290 \, ^\circC$. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.61 (s, 4H), 3.41 (s, 3H), 3.62 (t, $J = 5.6$ Hz, 4H), 4.14 (s, 2H), 4.25 (q, $J = 5.7$ Hz, 4H).

5.2.5 Synthesis of TR-MoEtPE

In a round bottom flask equipped with a magnetic stir bar, $N,N$-bis(2-hydroxyethyl)-3-methoxypropanamide (1.31 g, 6.84 mmol, 1 equiv.), succinic acid (808 mg, 6.84
mmol, 1 equiv.), and DPTS (800 mg, 2.74 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$
(12 mL, 2 mL / 1 mmol succinic acid) and purged with N$_2$ for 15 min with stirring. This
mixture was then briefly heated to reflux to homogenize the solution. The reaction was
cooled to 0 °C and DIC (3.2 mL, 21 mmol, 3 eq.) was added dropwise via syringe. The
reaction was allowed to come to room-temperature and stir for 116 h under N$_2$. The crude
reaction mixture was then diluted with CH$_2$Cl$_2$ and the diisopropyl urea byproduct was filtered
off. The dilute mixture was then concentrated under reduced pressure and purified via pre-
cipitation into cold 20:80 MeOH:iPrOH (2 x 200 mL). The purified polymer was dried
under reduced pressure to obtain the polymer as an elastic transparent solid. The resultant
polyester was characterized by SEC, DSC, and NMR. Recovered = 1.40 g, $M_n$ = 75.4 kDa,
$D_M$ = 1.47, $T_g$ = -13 °C, $T_{deg}$ = 294 °C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.61 (d, $J$ = 7.0 Hz,
7H), 3.33 (s, 3H), 3.70-3.61 (m, 6H), 4.23 (d, $J$ = 3.4 Hz, 4H).

5.3 General Procedure for Polyesterification of N-Substituted Amide Diol Monomers

![Polyesterification Reaction](image)

Polyesters were prepared according to previously established methods.$^9$ Since the N-
substituted amide diol monomers, polyesters, DPTS, DIC urea byproducts all displayed
similar solubilities in common organic solvents, the polymers were purified via dialysis against MeOH.

5.3.1 Synthesis of TR-cPrAPE

In a round bottom flask equipped with a magnetic stir bar, $N^1$-cyclopropyl-$N^4,N^4$-bis(2-hydroxyethyl)succinamide (1.86 g, 7.60 mmol, 1 equiv.), succinic acid (898 mg, 7.60 mmol, 1 equiv.), and DPTS (890 mg, 3.04 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (7.0 mL, 1 mL / 100 mmol succinic acid) and purged with N$_2$ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (3.6 mL, 23 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 86 h under N$_2$. The crude reaction mixture was diluted with CH$_2$Cl$_2$ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 1.74 g, $M_n$ = 25.5 kDa, $D_M$ = 1.66, $T_g$ = 14 °C, $T_{deg}$ = 274 °C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 0.47 (s, 2H), 0.70 (q, $J$ = 6.1 Hz, 2H), 2.45 (s, 2H), 2.71-2.59 (m, 7H), 3.67-3.58 (m, 4H), 4.23 (dt, $J$ = 16.0, 5.3 Hz, 4H), 6.67 (s, 1H).

5.3.2 Synthesis of TR-PyrAPE

In a round bottom flask equipped with a magnetic stir bar, $N,N$-bis(2-hydroxyethyl)-4-oxo-4-(pyrrolidin-1-yl)butanamide (1.25 g, 4.85 mmol, 1 equiv.), succinic acid (573 mg,
2.85 mmol, 1 equiv.), and DPTS (570 mg, 1.94 mmol, 0.4 equiv.) were dissolved in dry CH₂Cl₂ (4.5 mL, 1 mL / 100 mmol succinic acid) and purged with N₂ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (2.28 mL, 14.6 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 86 h under N₂. The crude reaction mixture was diluted with CH₂Cl₂ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 1.18 g, $M_n = 54.2$ kDa, $D_M = 1.60$, $T_g = 0$ °C, $T_{deg} = 307$ °C. $^1$H NMR (500 MHz, CDCl₃): $\delta$ 1.89 (dquintet, $J = 55.3$, 6.8 Hz, 4H), 2.65-2.59 (m, 5H), 2.71 (t, $J = 6.5$ Hz, 2H), 3.48-3.41 (m, 4H), 3.65 (dt, $J = 47.5$, 5.6 Hz, 4H), 4.28-4.20 (m, 4H).

5.3.3 Synthesis of TR-dEtAPE

In a round bottom flask equipped with a magnetic stir bar, $N^1,N^1$-diethyl-$N^4,N^4$-bis(2-hydroxyethyl)succinamide (512 mg, 1.97 mmol, 1 equiv.), succinic acid (232 mg, 1.97 mmol, 1 equiv.), and DPTS (230 mg, 0.787 mmol, 0.4 equiv.) were dissolved in dry CH₂Cl₂ (2 mL, 1 mL / 100 mg succinic acid) and purged with N₂ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (0.92 mL, 5.9 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 88 h under N₂. The crude reaction mixture was diluted with CH₂Cl₂ and the diisopropyl urea byproduct was
filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 519 mg, $M_n = 47.1$ kDa, $D_M = 1.66$, $T_g = -4 ^\circ$C, $T_{deg} = 304 ^\circ$C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.14 (dt, $J = 55.9$, 7.1 Hz, 6H), 2.70-2.60 (m, 7H), 3.35 (qd, $J = 7.1$, 2.3 Hz, 4H), 3.65 (dt, $J = 48.3$, 5.6 Hz, 4H), 4.23 (dt, $J = 26.0$, 5.2 Hz, 4H).

5.3.4 Synthesis of TR-iPrAPE

In a round bottom flask equipped with a magnetic stir bar, $N^1,N^1$-bis(2-hydroxyethyl)-$N^4$-isopropylsuccinamide (2.29 g, 9.30 mmol, 1 equiv.), succinic acid (1.10 g, 9.30 mmol, 1 equiv.), and DPTS (1.09 g, 3.72 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (8.4 mL, 1 mL / 100 mg succinic acid) and purged with N$_2$ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (4.4 mL, 27.9 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 86 h under N$_2$. The crude reaction mixture was diluted with CH$_2$Cl$_2$ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 1.82 g, $M_n = 56.5$ kDa, $D_M = 1.57$, $T_g = 10 ^\circ$C, $T_{deg} = 287 ^\circ$C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.12 (d, $J = 6.6$ Hz, 6H), 2.47 (t, $J$
= 6.6 Hz, 2H), 2.64-2.59 (m, 4H), 2.71-2.69 (t, 2H), 3.59 (t, J = 4.9 Hz, 2H), 3.66 (t, J = 5.4 Hz, 2H), 4.01 (dq, J = 13.0, 6.5 Hz, 1H), 4.23 (dt, J = 11.3, 5.5 Hz, 4H), 6.24-6.14 (m, 1H).

5.3.5 Synthesis of TR-nPrAPE

In a round bottom flask equipped with a magnetic stir bar, \(N^1, N^1\)-bis(2-hydroxyethyl)-\(N^4\)-propylsuccinamide (1.89 g, 7.66 mmol, 1 equiv.), succinic acid (904 mg, 7.66 mmol, 1 equiv.), and DPTS (900 mg, 3.06 mmol, 0.4 equiv.) were dissolved in dry \(CH_2Cl_2\) (6.9 mL, 1 mL / 100 mg succinic acid) and purged with \(N_2\) for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (3.6 mL, 23.0 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 86 h under \(N_2\). The crude reaction mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (3.6 mL, 23.0 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 86 h under \(N_2\). The crude reaction mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 2.11 g, \(M_n = 29.1\) kDa, \(D_M = 1.52\), \(T_g = 5^\circ C\), \(T_{deg} = 271^\circ C\). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 0.90 (t, \(J = 7.4\) Hz, 3H), 1.49 (q, \(J = 7.3\) Hz, 2H), 2.50 (t, \(J = 6.0\) Hz, 2H), 2.63-2.59 (m, 4H), 2.71 (t, \(J = 5.4\) Hz, 2H), 3.16 (q, \(J = 6.6\) Hz, 2H), 3.67-3.59 (m, 4H), 4.23 (dt, \(J = 12.6, 6.0\) Hz, 4H), 6.41 (s, 1H).
5.3.6 Synthesis of TR-bMoEtAPE

In a round bottom flask equipped with a magnetic stir bar, $N^1,N^1$-bis(2-hydroxyethyl)-$N^4,N^4$-bis(2-methoxyethyl)succinamide (1.33 g, 4.56 mmol, 1 equiv.), succinic acid (538 mg, 4.56 mmol, 1 equiv.), and DPTS (533 mg, 1.82 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (4.5 mL, 1 mL / 100 mg succinic acid) and purged with N$_2$ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (2.2 mL, 13.7 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 90 h under N$_2$. The crude reaction mixture was diluted with CH$_2$Cl$_2$ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 1.12 g, $M_n = 113.5$ kDa, $D_M = 1.58$, $T_g = -20$ °C, $T_{deg} = 315$ °C. $^1$H NMR (300 MHz; CDCl$_3$): $\delta$ 2.66 (dq, $J = 20.0$, 6.2 Hz, 8H), 3.31 (d, $J = 7.0$ Hz, 6H), 3.70-3.47 (m, 12H), 4.22 (dt, $J = 13.5$, 6.3 Hz, 4H).

5.3.7 Synthesis of TR-MorAPE

In a round bottom flask equipped with a magnetic stir bar, $N,N$-bis(2-hydroxyethyl)-4-morpholino-4-oxobutanamide (450 mg, 1.64 mmol, 1 equiv.), succinic acid (194 mg, 1.64 mmol, 1 equiv.), and DPTS (192 mg, 0.66 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (3 mL, 2 mL / 1 mmol succinic acid) and purged with N$_2$ for 15 min with magnetic stirring.
This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (0.77 mL, 4.9 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 99 h under N\textsubscript{2}. The crude reaction mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2} and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure and purified via precipitation into cold 20:80 MeOH:iPrOH (2 x 200 mL). The purified polymer was dried under reduced pressure to obtain the polymer as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 486 mg, \( M_n = 31.4 \text{ kDa}, D_M = 1.35, T_g = 4 \text{ °C}, T_{\text{deg}} = 306 \text{ °C} \). \( ^1\text{H NMR (300 MHz, CDCl}_3\): \( \delta \) 2.66 (ddd, \( J = 22.0, 9.6, 5.9 \text{ Hz, 8H}), 3.71-3.51 (m, 12H), 4.29-4.20 (m, 4H).

5.3.8 Synthesis of TR-MoEtAPE

In a round bottom flask equipped with a magnetic stir bar, \( N^1,N^1\)-bis(2-hydroxyethyl)-\( N^4\)-(2-methoxyethyl)succinamide (760 mg, 2.90 mmol, 1 equiv.), succinic acid (343 mg, 2.90 mmol, 1 equiv.), and DPTS (339 mg, 1.16 mmol, 0.4 equiv.) were dissolved in dry CH\textsubscript{2}Cl\textsubscript{2} (7 mL, 2 mL / 1 mmol succinic acid) and purged with N\textsubscript{2} for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (1.4 mL, 8.7 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 69 h under N\textsubscript{2}. The crude reaction mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2} and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure and purified via precipitation into cold 20:80 MeOH:iPrOH (2 x 200 mL). The purified polymer was dried under reduced pressure to obtain the polymer as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 153
mg, $M_n = 43.7$ kDa, $D_M = 1.39$, $T_g = -8 \, ^\circ C$, $T_{deg} = 266 \, ^\circ C$. $^1\text{H}$ NMR (500 MHz, CDCl$_3$): $\delta$

1.18 (t, $J = 7.0$ Hz, 3H), 2.52 (t, $J = 6.5$ Hz, 2H), 2.64-2.59 (m, 4H), 2.71 (t, $J = 6.4$ Hz, 2H), 3.40 (q, $J = 5.3$ Hz, 2H), 3.51-3.46 (m, 4H), 3.59 (t, $J = 4.5$ Hz, 2H), 3.66 (t, $J = 5.7$ Hz, 2H), 4.23 (dt, $J = 12.7$, 6.1 Hz, 4H), 6.43 (dd, $J = 17.0$, 5.4 Hz, 1H).

5.3.9 Synthesis of TR-EoEtAPE

In a round bottom flask equipped with a magnetic stir bar, $N^1$-(2-ethoxyethyl)$-N^4,N^4$-bis(2-hydroxyethyl)succinamide (564 g, 2.15 mmol, 1 equiv.), succinic acid (254 mg, 2.15 mmol, 1 equiv.), and DPTS (251 mg, 0.86 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (4 mL, 1 mL / 1 mmol succinic acid) and purged with N$_2$ for 15 N$_2$ with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 $^\circ$C and DIC (1.0 mL, 6.5 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 65 h under N$_2$. The crude reaction mixture was diluted with CH$_2$Cl$_2$ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 360 mg, $M_n = 30.3$ kDa, $D_M = 1.38$, $T_g = 9 \, ^\circ C$, $T_{deg} = 273 \, ^\circ C$. $^1\text{H}$ NMR (300 MHz, CDCl$_3$): $\delta$

1.19 (t, $J = 7.0$ Hz, 3H), 2.53 (t, $J = 6.5$ Hz, 2H), 2.62 (d, $J = 14.4$ Hz, 5H), 2.71-2.69 (m, 2H), 3.40 (d, $J = 5.4$ Hz, 2H), 3.50-3.46 (m, 4H), 3.66 (t, $J = 5.7$ Hz, 4H), 4.23 (q, $J = 6.5$ Hz, 4H), 6.42 (s, 1H).
5.3.10 Synthesis of TR-THFAPE

In a round bottom flask equipped with a magnetic stir bar, $N^1,N^1$-bis(2-hydroxy-ethyl)-$N^4$-((tetrahydrofuran-2-yl)methyl)succinamide (314.1 mg, 1.089 mmol, 1 equiv.), succinic acid (127.4 mg, 1.078 mmol, 1 equiv.), and DPTS (127 mg, 0.436 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (3 mL, 1 mL / 100 mmol COOH) and purged with N$_2$ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (0.51 mL, 3.27 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 80 h under N$_2$. The crude reaction mixture was diluted with CH$_2$Cl$_2$ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 187.4 mg, $M_n = 48.5$ kDa, $D_M = 1.49$, $T_g = 13$ °C, $T_{deg} = 275$ °C.

5.3.11 Synthesis of TR-PropargylAPE

In a round bottom flask equipped with a magnetic stir bar, $N^1,N^1$-bis(2-hydroxy-ethyl)-$N^4$-(prop-2-yn-1-yl)succinamide (1.02 g, 4.23 mmol, 1 equiv.), succinic acid (499 mg, 4.23 mmol, 1 equiv.), and DPTS (494 mg, 1.69 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (9 mL, 2 mL / 1 mg succinic acid) and purged with N$_2$ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (1.3 mL, 12.7 mmol, 3 eq.) was added dropwise via
syringe. The reaction was allowed to come to room-temperature and stir for 87 h under N₂. The crude reaction mixture was diluted with CH₂Cl₂ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure and purified via precipitation into cold 20:80 MeOH:iPrOH (2 x 200 mL). The purified polymer was dried under reduced pressure to obtain the polymer as a yellow amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 445 mg, $M_n = 12.9$ kDa, $D_M = 1.33$, $T_g = 13$ °C, $T_{deg} = 242$ °C. $^1$H NMR (300 MHz, CDCl₃): δ 2.76-2.56 (m, 9H), 3.64 (dt, $J = 18.6$, 4.8 Hz, 4H), 4.01 (t, $J = 2.5$ Hz, 2H), 4.26 (t, $J = 5.6$ Hz, 4H), 7.02 (s, 1H). $^1$H NMR (300 MHz, DMSO-d₆): δ 2.32 (t, $J = 6.8$ Hz, 2H), 2.57-2.48 (m, 6H), 3.02 (t, $J = 2.3$ Hz, 1H), 3.60-3.45 (m, 4H), 3.81 (dd, $J = 5.4$, 2.4 Hz, 2H), 4.11 (dt, $J = 30.3$, 5.3 Hz, 4H), 8.19 (t, $J = 5.5$ Hz, 1H).

5.3.12 Synthesis of TR-PPDAPE

In a round bottom flask equipped with a magnetic stir bar, $N,N$-bis(2-hydroxyethyl)-4-oxo-4-(piperidin-1-yl)butanamide (1.00 g, 3.68 mmol, 1 equiv.), succinic acid (435 mg, 3.68 mmol, 1 equiv.), and DPTS (430 mg, 1.47 mmol, 0.4 equiv.) were dissolved in dry CH₂Cl₂ (8 mL, 2 mL / 1 mmol succinic acid) and purged with N₂ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (1.73 mL, 11.1 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 80 h under N₂. The crude reaction mixture was diluted with CH₂Cl₂ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced
pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 572 mg, $M_n = 26.0$ kDa, $D_M = 1.40$, $T_g = 22 {^\circ}C$, $T_{deg} = 309 {^\circ}C$. $^1$H NMR (300 MHz, CDCl$_3$): δ 1.63-1.51 (m, 6H), 2.62 (dd, $J = 6.9$, 6.3 Hz, 4H), 2.68 (s, 4H), 3.53-3.42 (m, 4H), 3.65 (dt, $J = 28.0$, 5.7 Hz, 4H), 4.29-4.20 (m, 4H).

5.3.13 Synthesis of TR-dnBuAPE

In a round bottom flask equipped with a magnetic stir bar, $N^1,N^1$-dibutyl-$N^4,N^4$-bis(2-hydroxyethyl)succinamide (938 mg, 2.97 mmol, 1 equiv.), succinic acid (350 mg, 2.97 mmol, 1 equiv.), and DPTS (347 mg, 1.19 mmol, 0.4 equiv.) were dissolved in dry CH$_2$Cl$_2$ (7 mL, 2 mL / 1 mmol succinic acid) and purged with N$_2$ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (1.40 mL, 8.90 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 80 h under N$_2$. The crude reaction mixture was diluted with CH$_2$Cl$_2$ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 641 mg, $M_n = 44.5$ kDa, $D_M = 1.42$, $T_g = -4 {^\circ}C$, $T_{deg} = 315 {^\circ}C$. $^1$H NMR (300 MHz, CDCl$_3$): δ 0.92 (dt, $J = 12.8$, 7.2 Hz, 6H), 1.36-1.24 (m, 4H), 1.60-1.45 (m, 4H), 2.65 (ddd, $J = 13.6$, 10.2, 5.8 Hz, 8H), 3.27 (q, $J = 7.5$ Hz, 4H), 3.71-3.58 (m, 4H), 4.27-4.19 (m, 4H).
5.3.14 Synthesis of TR-HepAPE

In a round bottom flask equipped with a magnetic stir bar, ethyl 4-(heptylamino)-4-oxobutanoate (857 mg, 2.83 mmol, 1 equiv.), succinic acid (335 mg, 2.83 mmol, 1 equiv.), and DPTS (331 mg, 1.13 mmol, 0.4 equiv.) were dissolved in dry CH₂Cl₂ (6 mL, 1 mL / 1 mmol succinic acid) and purged with N₂ for 15 min with magnetic stirring. This mixture was then briefly heated to reflux to homogenize the solution. The reaction was cooled to 0 °C and DIC (1.33 mL, 8.50 mmol, 3 eq.) was added dropwise via syringe. The reaction was allowed to come to room-temperature and stir for 80 h under N₂. The crude reaction mixture was diluted with CH₂Cl₂ and the diisopropyl urea byproduct was filtered off. The dilute mixture was then concentrated under reduced pressure, solvated in a minimal amount of MeOH, and further purified via dialysis against MeOH for 24 h with solvent changes at 3 h, 6 h, and 16 h. The dialysis mixture was dried under reduced pressure to obtain pure polyester as a white amorphous solid. The resultant polyester was characterized by SEC, DSC, TGA, and NMR. Recovered = 712 mg, $M_n = 56.8$ kDa, $D_M = 1.34$, $T_g = 9$ °C, $T_{deg} = 277$ °C. $^1$H NMR (300 MHz, CDCl₃): δ 0.87 (q, $J = 4.2$ Hz, 3H), 1.27 (d, $J = 3.3$ Hz, 8H), 1.49-1.45 (m, 2H), 2.52-2.47 (m, 2H), 2.61 (d, $J = 13.4$ Hz, 4H), 2.74-2.70 (m, 2H), 3.22-3.16 (m, 2H), 3.68-3.58 (m, 4H), 4.26-4.20 (m, 4H), 6.35 (s, 1H).
CHAPTER VI

FIRST GENERATION THERMORESPONSIVE POLYESTERS

6.1 Introduction

In 2013, Gokhale and Xu from our laboratory reported the synthesis and characterization of a library of biodegradable “peptide-like” poly(ester)s (PEs). Their goal was to create new synthetic poly(peptide) analogues based on PEs, similar to poly(N-substituted glycine)s (also known as poly(α-peptoids)) to address the lack of functionality inherent to many PE biomaterials. The “peptide-like” PEs were designed with a significant amount of oxygen and nitrogen atoms in the polymer backbone (similar to poly(peptide)s) as to increase their hydrophilicity and make them better able to interact with complex biological systems. This was achieved by polymerizing succinic acid (SA) with N-substituted diethanolamine (DEA) bearing a variety of bio-inspired orthogonal pendant groups which allowed for covalent attachment of biologically relevant ligands.

In the previous work, room-temperature carbodiimide-mediated polyesterification was used instead of traditional high-temperature polycondensation for a number of reasons. The lack of side-reactions in the polymerization (such as transesterification and cyclization) allowed for the generation of high molecular weight (< 100 kDa) PEs. Additionally, the use of the more “gentle” carbodiimide polyesterification as opposed to high
temperature polycondensation permitted the use of $N$-substituted diol monomers containing delicate functional groups. The modular synthetic route allowed for the synthesis of statistical copolymers containing a variety of different pendant groups, giving the “peptide-like” PEs tunable physical properties. Despite the relatively hydrophilic backbone, the “peptide-like” PEs did not display room-temperature aqueous solubility or thermoresponsive behavior.

As discussed in Chapter II, the design of novel Lower Critical Solution Temperature (LCST) type biodegradable materials has become an area of intense research due to the promise of next-generation applications requiring such materials as well as the current lack of synthetic examples.

![Chemical structures of polymers](image)

**Figure 6.1:** Chemical structure of poly(acrylamide)s (PAMs), poly(alkyloxazoline)s (PAOxs), elastin-like poly(peptide)s (ELPs), “peptide-like” poly(ester)s, and thermoresponsive poly(ester)s (TR-PE, this work).

**Figure 6.2:** Select thermoresponsive PAOXS and PAMs as the inspiration for TR-PE library.
The present chapter details our efforts toward designing a novel modular thermoresponsive PE (TR-PE) system that exhibits LCST-type behavior and biodegradation. Owing to the structural similarities between our previous “peptide-like” PEs and PAMs, PAOxs, and ELPs (Figure 6.1), we hypothesized that PEs with specific pendant groups would exhibit thermoresponsive behavior. We designed a series of PAOx and PAM pendant group mimics in order to modulate the hydrophilicity and resulting \( T_{cp} \) (Figure 6.2). Similar to other coacervate forming thermoresponsive polymers such as ELPs\(^3\) and poly(phosphoester)s,\(^2\) we hypothesized that the multiple polar hydrogen bonding sites in the PEs backbone would prevent complete dehydration of the PEs leading to thermoresponsive coacervate formation. Finally, we predicted that ester bonds present in the PEs backbone would demonstrate similar degradation kinetics as observed in our previous “peptide-like” PEs.

6.2 Synthesis and Characterization of Monomers and Polyesters

It is well-known that the poly(2-alkyl-oxazoline)s PiPOx and PnPrOx exhibit LCST behavior at 36 and 24 °C, respectively.\(^{5,205}\) Given the structural similarities of PAOx to our “peptide-like” PEs, we initially synthesized two \( N,N \)-bis(hydroxyethyl) alkyl amide (HEA) PEs containing \( i \)-propyl and \( n \)-propyl functional groups using room-temperature carbodiimide mediated polymerization as shown in Scheme 6.1.\(^{184}\) Previous work by our lab has shown that this method allows for the synthesis of high molecular weight PEs with narrow PDI in high yield.\(^9\) However, these PEs proved to be insoluble in aqueous solutions even after 16 h at 4 °C. As such, it was assumed that the hydrophobic/hydrophilic balance of the PE was not optimal to elicit a temperature-sensitive response.
In order to increase the hydrophilicity of the PEs, a set of five $N,N$-bis(hydroxy-ethyl) $N$-(alkyl)succinamide (HESA) monomers inspired by various thermoresponsive PAMs were synthesized. Despite the lower functional group density of the PEs as compared to PAMs, it was believed that the more hydrophilic pendant groups would impart temperature-sensitive solubility to the resultant TR-PEs as compared to the PAOx-like HEA-PEs. As the LCST of each PAM is affected by the hydrophobicity of the amide, it was hypothesized that different acrylamide mimics would display varying thermoresponsivity in a similar pattern. The desired amine was first reacted with ethyl succinyl chloride to produce the corresponding succinamide ester in quantitative yields. A transamidation reaction in neat DEA afforded the pure HESA monomer after silica gel flash chromatography.

HESA monomers were then polymerized using room-temperature carbodiimide-mediated polyesterification. Owing to the similar solubilities of the monomers, PEs, DPTS, and DIC urea byproducts in common solvents, purification by precipitation proved unsuccessful. Instead, pure TR-PEs were obtained by dialysis against MeOH at room-temperature for 24 h and drying under reduced pressure (Figure 6.3). The resultant PEs were characterized by NMR, which proved the removal of DPTS and urea byproducts. Adjusting the stoichiometry of diol to diacid in the polymerization resulted in a variety of molecular weight TR-PEs that were analyzed via SEC. TR-PEs were categorized according to the $N$-substituent; for example, the TR-PE bearing an $N$-$i$-propylamide (iPrA) pendant is designated TR-iPrAPE.
Scheme 6.1: Synthetic Route for the Preparation of PEs. Reagents and conditions: (i) DEA, neat, 80 °C, 16 h; (ii) SA, DIC, DPTS, CH₂Cl₂, 0 °C to room-temperature, 48 h; (iii) ethylsuccinyl chloride, Et₃N, CH₂Cl₂, 0 °C to room-temperature, 1 h.

6.3 Thermally Induced Phase Transitions

Aqueous solutions (10.0 mg/mL) of TR-PEs were prepared by equilibrating the PE in DI water overnight at 4 °C. High molecular weight (~55 kDa) TR-iPrAPE, TR-dEtAPE, and TR-PyrAPE were water-soluble at low temperatures and showed a rapid increase in turbidity upon being brought to room-temperature (Figure 6.4). Cloudy solutions of these PEs could be returned to their initial transparent state upon cooling. Despite their lower molecular weights (~25 kDa), TR-cPrAPE and TR-nPrAPE were not completely soluble in aqueous conditions at 0 °C.
The thermoresponsivity of aqueous TR-PE solutions was quantified using UV–vis and \(^1\)H NMR. To minimize molecular weight influences, TR-PEs of similar high molecular weight were chosen for comparative analysis. As shown in Figure 6.4, a sharp reversible \(T_{cp}\) was observed for three TR-PEs of similar molecular weight: TR-iPrAPE (7.8 °C), TR-dEtAPE (11.9 °C), and TR-PyrAPE (15.8 °C). As hypothesized, the \(T_{cp}\) was affected by amide side chain identity. However, the \(T_{cp}\) trend for TR-PEs did not seem to follow that for PAMs (Figure 6.2), as TR-cPrAPE was expected to have a \(T_{cp}\) near that of TR-PyrAPE but was instead insoluble. Similarly, TR-dEtAPE and TR-iPrAPE were expected to have comparable \(T_{cp}\) but instead showed a 4.1 °C difference. These variations will be discussed in further detail below. Compared to thermoresponsive PAMs and PAOxs, TR-PEs exhibit a more significant hysteresis with TR-iPrAPE being unable to fully rehydrate within the experimental time frame. An increase in hysteresis is commonly observed when intra- and intermolecular hydrogen bonds form in the dehydrated state, making rehydration more difficult.\(^74\) Upon collapse of the PE chains, it is likely that some water molecules remain in the dehydrated chains and act as bridge points between the many hydrogen bond acceptors present in the TR-PEs. This suggests a more inefficient dehydration, such as a coacervate-type response, takes place above the LCST. Furthermore, the hydrogen bond donating amide of TR-iPrAPE can also form intra- and intermolecular hydrogen bonds, increasing the hysteresis as compared to TR-dEtAPE and TR-PyrAPE. It has been reported that the self-assembly of complex structures such as \(\beta\)-turns can increase hysteresis by up to 20 °C in the case of certain ELPs.\(^{123}\) Preliminary attempts at determining if the TR-PEs exhibit any ordered structures were carried out by analysis of dilute TR-PE solutions. However, these
studies did not show any distinguishing circular dichroism (CD) signals and the lack of any chiral center in the PEs may preclude any ordered structures for these PEs.

Figure 6.3: TR-PE library.

Figure 6.4: Reversible cloud point behavior of TR-PyrAPE (top, $T_{cp} = 15.8^\circ C$, 10 mg/mL) and temperature-dependent transmittance of TR-iPrAPE, TR-dEtAPE, and TR-PyrAPE (bottom, $M_n \sim 55$ kDa, 10 mg/mL, 1 °C/min) in DI water exhibiting clear hysteresis.
Figure 6.5: Variable temperature $^1$H NMR spectra of TR-PyrAPE ($T_{cp} = 15.8$ °C) in D$_2$O above and below cloud point.

Variable temperature $^1$H NMR in D$_2$O was used to examine the change in PE hydration with temperature. A representative example of TR-PyrAPE is shown in Figure 6.5. Above the $T_{cp}$ at 20 °C, the peaks corresponding to the DEA backbone begin to decrease in intensity and shift from two to three peaks as dehydration occurs. Moreover, the pyrrolidinyl alkyl peaks are seen to decrease in intensity and transform. Unlike thermoresponsive polymers which undergo efficient dehydration, no peaks are observed to completely disappear, indicating partial dehydration and a coacervate-type response. Changes in peak intensity and shape are also observed above the $T_{cp}$ of TR-iPrAPE and TR-dEtAPE (see Appendix).

It has been well established that the presence of an endothermic peak in a DSC measurement is one of the most accurate and robust methods for determining the LCST of thermoresponsive polymer solutions. The endothermic peak appears at the LCST when hydrogen bonds between structured water molecules and the polymer break, resulting in a
large gain of entropy that compensates for the loss of entropy incurred by the dehydrated polymer.  

However, no endothermic peak was observed for any TR-PE solution even at high concentrations (150 mg/mL) despite showing a clear cloud point transition. This behavior is indicative of coacervate-type polymers, in which the partial dehydration of fewer structured waters leads to a reduced amount of entropy gained (relative to coil–globule polymers).  

The loss of entropy due to collapse of the polymer chain cannot be overcome by smaller entropy gained from partial dehydration of structured water molecules, leading to reduction or elimination of the endothermic peak. The absence of an endothermic peak in DSC strongly suggested that TR-PEs were coacervate-type polymers.

Table 6.1: Characterization of TR-PEs

<table>
<thead>
<tr>
<th>Polyester</th>
<th>$M_n$ (kDa)</th>
<th>$M_w$ (kDa)</th>
<th>$D_M$</th>
<th>$T_g$ (°C)</th>
<th>$T_{cp}$ (°C)</th>
<th>Coacervate [PE] (%)</th>
<th>Coacervate droplet $R_h$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-cPrAPE</td>
<td>25.5</td>
<td>42.4</td>
<td>1.6</td>
<td>13.6</td>
<td>N/A</td>
<td>53</td>
<td>64.6</td>
</tr>
<tr>
<td>TR-nPrAPE</td>
<td>29.1</td>
<td>43.9</td>
<td>1.5</td>
<td>4.8</td>
<td>N/A</td>
<td>20</td>
<td>65.4</td>
</tr>
<tr>
<td>TR-iPrAPE</td>
<td>56.5</td>
<td>88.5</td>
<td>1.6</td>
<td>10.0</td>
<td>7.8</td>
<td>32</td>
<td>61.4</td>
</tr>
<tr>
<td>TR-dEtAPE</td>
<td>56.5</td>
<td>87.6</td>
<td>1.5</td>
<td>-4.2</td>
<td>11.9</td>
<td>33</td>
<td>57.3</td>
</tr>
<tr>
<td>TR-PyrPE</td>
<td>54.2</td>
<td>86.6</td>
<td>1.5</td>
<td>0.2</td>
<td>15.8</td>
<td>51</td>
<td>44.2</td>
</tr>
</tbody>
</table>

aDetermined by DMF SEC relative to PMMA standards. b Determined by DSC. c Defined as 50% transmission during temperature controlled UV–vis analysis. d Corresponding values of thermoresponsive PAMs. e Average of three measurements. f Determined using temperature controlled DLS analysis.

6.4 Effect of Polyester Concentration, Molecular Weight, and Cosolutes on Phase Transition

In order to further explore the aqueous solution properties of the TR-PE system, UV–vis was used to probe the effect of PE concentration, molecular weight, and cosolutes on $T_{cp}$. As expected, increasing PE concentration increases the PE available to form coacervates and results in an earlier onset of $T_{cp}$ (Figure 6.6A) similar to what is seen for other thermoresponsive systems.  

Increasing the molecular weight of TR-PEs decreases
their solubility, in turn logarithmically decreasing the $T_{cp}$. This behavior is similar to what is observed with more monodisperse ELPs (Figure 6.6B). Increasing the concentration of hydrogen bond disrupting NaCl is well-known to promote thermoresponsive polymer collapse\(^{21,24}\) ("salting out" effect) and was observed to decrease the $T_{cp}$ in a linear fashion (Figure 6.6C). Conversely, increasing the amount of SDS surfactant, which stabilizes the hydrated PE, was observed to raise the $T_{cp}$ (Figure 6.6D), in agreement with previous literature precedent.\(^{3,28,207}\) Furthermore, it was noted that higher concentrations of SDS were able to solubilize TR-cPrAPE and TR-nPrAPE which allowed for a temperature response.

Figure 6.6: Aqueous solution properties of TR-PEs (10 mg/mL unless otherwise stated) as a function of concentration (A), molecular weight (B), NaCl (C), and SDS (D), and urea (E).

Poly($N$-isopropylacrylamide) (PNIPAM) has been previously used as a model to investigate the mechanism of protein denaturation by urea.\(^{22}\) Despite numerous investigations, this mechanism is still not well understood. The addition of urea results in a decrease
of the LCST of PNIPAM, which is hypothesized to be the result of urea interacting bivalently with the polymer and stabilizing its dehydrated state via intra- and intermolecular hydrogen bonding.\textsuperscript{71-72} An opposite effect is observed for ELPs in which the LCST increases with urea concentration, as the hydrated state is made more stable. The current TR-PE system was observed to display a linear increase in $T_{cp}$ with increasing urea content, similar to that of ELPs (Figure 6.6E). The resultant increase in thermoresponsive solubility was also seen for TR-cPrAPE and TR-nPrAPE. This behavior strongly indicates that despite the monomers being inspired by PAMs, in solution TR-PEs appear to behave similar to ELPs. In the future, TR-PEs may provide a better model to understand the role of urea in protein denaturation.

6.5 Tuning Phase Transition Temperature via Copolymerization

As TR-PE homopolymers displayed distinctive $T_{cp}$, it was believed that thermoresponsivity could be tuned by copolymerization of different monomers similar to other thermoresponsive PAMs,\textsuperscript{5, 67, 206} PEs,\textsuperscript{208} polyphosphoesters,\textsuperscript{163} and polyamides.\textsuperscript{120, 173-174, 209}

Table 6.2: Characterization of TR-PE Copolyesters

<table>
<thead>
<tr>
<th>monomer feed (iPr:Pyr)</th>
<th>PE composition</th>
<th>$M_n$ (kDa)</th>
<th>$D_M$</th>
<th>$T_{cp}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>100:0</td>
<td>56.6</td>
<td>1.6</td>
<td>7.8</td>
</tr>
<tr>
<td>75:25</td>
<td>71:29</td>
<td>52.2</td>
<td>1.4</td>
<td>7.9</td>
</tr>
<tr>
<td>50:50</td>
<td>48:52</td>
<td>56.0</td>
<td>1.4</td>
<td>9.5</td>
</tr>
<tr>
<td>25:75</td>
<td>27:73</td>
<td>55.0</td>
<td>1.4</td>
<td>13.1</td>
</tr>
<tr>
<td>0:100</td>
<td>0:100</td>
<td>54.2</td>
<td>1.5</td>
<td>15.8</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Determined by $^1$H NMR. \textsuperscript{b}Determined by DMF SEC relative to PMMA standards. \textsuperscript{c}Defined as 50% transmission during temperature controlled UV–vis analysis.
It has been previously established that the hydrophobic/hydrophilic balance can be used to control the behavior of thermoresponsive polymers. Within the realm of coacervation-type polymers, Sugihara et al. synthesized three types of poly(hydroxyethyl vinyl ether-co-isobutyl vinyl ether): random copolymers, diblock copolymers, and “block and random” copolymers. Of these, only the random copolymers exhibited a sharp phase separation above the $T_{cp}$, resulting in the formation of coacervate droplets. The authors suggested that the random sequence of the comonomers was crucial for well-defined phase separation and coacervate formation. Furthermore, Maeda et al. was able to show $T_{cp}$ control of poly(NIPAM-ran-HIPAM), a thermoresponsive coacervate-type copolymer, by controlling the comonomer content. Given that the random “peptide-like” copolyester composition as determined by NMR was statistically similar to the monomer feed, it was expected that the composition of TR-PES could be reasonably controlled by the monomer feed as well, resulting in control over the $T_{cp}$. As shown in Figure 6.7, statistically random TR-PES of similar high molecular weights display $T_{cp}$ between that of their respective homopolymers (Table 6.2), indicating that the temperature response can be tuned by monomer feed.
Coacervate Analysis

Upon being left at room-temperature overnight, the turbid aqueous solutions of TR-rPrAPE, TR-dErAPE, and TR-PyrAPE were observed to phase separate into a polymer-deficient aqueous phase and a viscous polymer-rich coacervate phase. As seen in Figure 6.9, optical microscopy of the turbid solutions showed submicron-sized coacervate droplets. Centrifugation of the turbid solutions allowed for rapid coalescence of the coacervate droplets and analysis of the PE concentration (Table 6.1). Following the trend witnessed in the $T_{cp}$ experiments, polymer coacervate concentration did not seem to correlate with the hydrophobicity of the amide, which will be discussed in more detail below. The $z$-average hydrodynamic radius ($R_{h,\text{app}}$) of TR-PE coacervate droplets was quantified using DLS.

As shown in a representative CONTIN analysis of the DLS measurements of TR-dEtAPE (Figure 6.8), the onset of coacervate formation is observed above the $T_{cp}$. Coacervate droplets are shown to be relatively monodisperse and stable for at least 3 h, making
them potentially useful for a number of biomaterials applications such as controlled delivery of therapeutics. It should be noted that there is small difference between the onset of coacervate formation, as obtained from DLS measurements, and $T_{cp}$. This is primarily due the qualitative nature of $T_{cp}$ experiments, which measure macroscopic changes and are affected by conditions such as concentration and heating rate. The results of DLS analysis show that the higher the $T_{cp}$ and coacervate water concentration, the greater the size of the resulting coacervate droplets (Table 6.1, Appendix). Coacervates droplets of TR-rPrAPE ($T_{cp} = 7.8 \, ^{\circ}C$) begin at $R_{h, app} = 100 \, \text{nm}$, while those of TR-PyrAPE ($T_{cp} = 15.8 \, ^{\circ}C$) show an initial size of $R_{h, app} = 220 \, \text{nm}$. Based on the $T_{cp}$ and coacervate data, it seems that the type of amide present on the TR-PE side chain plays a greater effect on the solution properties than originally hypothesized. For all three secondary amide-based TR-PEs, the $T_{cp}$ is much lower than would be expected if only hydrophilicity was taken into account. The tertiary amide-based TR-PEs show better solubility, higher coacervate water content, and less hysteresis than the secondary amide-based TR-PEs. This difference is likely due to the intra- and intermolecular hydrogen bonding available to the secondary amide side chain (a hydrogen bond donor and acceptor) and the numerous hydrogen bond accepting oxygen and nitrogen atoms per repeat unit as compared to the tertiary amide side chains (only a hydrogen bond acceptor). As previously discussed, such hydrogen bonding is known to increase the hysteresis of thermoresponsive polymers and makes the resolvation of the polymers less favorable.
in order to show the feasibility of TR-PE coacervates for the thermally induced encapsulation of desirable biologic compounds, Nile Red was used as a model. Nile Red is a hydrophobic dye with limited water solubility. A drop of 50 mM Nile Red in DMSO was added to a solution of TR-dEtAPE, which was quickly brought to room-temperature.

Upon coacervation and centrifugation, the purple dye-rich coacervate phase is easily observable (see Appendix). In contrast, no such behavior was observed when an aliquot of Nile Red was added to a blank solution. As seen in Figure 6.9, the addition of Nile Red caused only the Nile Red swollen coacervates to emit a red fluorescence, indicating successful incorporation of the dye. Our lab is currently studying the encapsulation efficiency and release kinetics of the TR-PE system for more complex hydrophilic model drugs and proteins.
6.8 Degradation Behavior

The PE backbone of the TR-PE system possesses inherent hydrolytic degradability. This is a significant advantage for numerous biomedical applications as compared to nondegradable thermoresponsive polymers such as PNIPAM or the enzymatically cleavable backbone of poly(amino acids) like ELPs. As a model study, the degradation of high molecular weight TR-dEtAPE was monitored at 37 °C. Over a period of 7 days, TR-dEtAPE degraded from 72.7 to 26.7 kDa, a 63% $M_n$ loss (Figure 6.10). This is comparable to the reported degradation of the more hydrophobic p(mAla) “peptide-like” PE, which degraded from 63.3 to 41.4 kDa (a 35% $M_n$ loss) over the same 7 day period, as well as for the degradation of thermoresponsive poly(MEMO/ME$_2$MO-alt-SA) PEs. Based on this evidence, it is reasonable to assume that the four other TR-PEs will exhibit similar degradation trends. It is likely that the formation of a polymer-rich coacervate phase reduced the rate of ester bond hydrolysis, resulting in a slower degradation than would occur with a completely soluble PE.
Figure 6.10. Hydrolytic degradation of TR-dEtAPE over a period of 7 days; n = 3.

6.9 Conclusion

In this work, we have developed a novel biodegradable PE system that exhibits reversible temperature-dependent coacervation. The high molecular weight PEs were synthesized using room-temperature carbodiimide-mediated condensation polymerization. The temperature of coacervation, as well as coacervate droplet size and polymer concentration, was observed to be dependent on the amide side chain structure. Although the monomers were inspired by PAMs, the increase in $T_{cp}$ of the PEs in the presence of urea indicate that their coacervation mechanism is likely similar to that of ELPs. Further evidence for the similarity of TR-PEs to ELPs was provided by the lack of thermal signals in DSC experiments, indicating an incomplete dehydration of the polymer phase. The modular architecture of the system allows for tuning of the thermoresponsivity through copolymerization as well as the possible incorporation of monomers capable of covalent attachment of physiologically useful ligands. This platform represents a significant step forward within the field of thermoresponsive biomaterials and shows promise as a system for drug or protein delivery, protein purification, and injectable scaffolds. By this method it would be possible
to deliver sensitive biomolecules by simple mixing with the soluble polymer phase and encapsulation by a temperature change. The biodegradable nature of the PEs theoretically allows for periodic delivery of therapeutics by this methodology.

6.10 Copyright notice

A significant part of this chapter was adapted with permission from “A Library of Thermoresponsive, Coacervate-Forming Biodegradable Polyesters,” originally published in *Macromolecules*. Copyright 2015 American Chemical Society.
CHAPTER VII

SECOND GENERATION THERMORESPONSIVE POLYESTERS

7.1 Introduction

As discussed in Chapter VI, our lab has investigated the modular synthesis of a variety of N-functionalized diol monomers to generate low \( T_g \) biodegradable “peptide-like” polyesters\(^9\) as well as low modulus thermoresponsive polyesters (TR-PEs).\(^{212}\) Interestingly, TR-PEs did not undergo complete and efficient dehydration when brought above their Lower Critical Solution Temperatures (LCSTs), but instead exhibited a liquid-liquid phase separation to form stable polymer-rich coacervates. As previously mentioned, coacervates are known to be ideal environments for locally segregating sensitive biomolecules\(^6\)\(^-\)\(^7\) and show great promise for next-generation biomedical applications such as protein purification,\(^{122}\) drug delivery,\(^{123}\) and tissue engineering.\(^{55,127}\) However, the cloud point temperature (\( T_{cp} \), often used as an approximation of the LCST) of all TR-PE homopolymers remained low in the range of 0 – 15.8 °C, limiting their possible biomedical applications especially if copolymerized with more hydrophobic monomers.

In this chapter, we describe our recent efforts to synthesize a new biodegradable, coacervate forming TR-PE with an increased LCST and explore its physical and thermal properties in detail. Ideally, the LCST should be significantly above body temperature (37
°C) so that the temperature-induced phase transition can be easily tuned by copolymerization. Our previous studies suggested that a hydrophilic tertiary amine would likely increase the LCST. In order to achieve this, we were inspired by the rarely studied poly(alkoxy-acrylamide) (PAOM), poly(N,N-bis(2-methoxyethyl)acrylamide), which displays an LCST ~54 °C.\textsuperscript{46,48} Bis-2-methoxyethyamine (bMoEtA) contains two alkoxy groups and was chosen as a starting tertiary amine to generate a hydrophilic diol monomer. The resulting high $M_n$ TR-PE, TR-bMoEtAPE, displayed a threefold increase in LCST as compared to the previous TR-PEs as well as a decrease in $T_g$. Thermoreversibility was observed to be nearly constant over a series of cycles and was affected by Hofmeister series anions, PE concentration, and comonomer feed ratio. TR-bMoEtAPE exhibited similar coacervation behavior as observed with previous TR-PEs and hydrolytically degraded over a period of seven days. Additionally, preliminary in vivo studies indicated that TR-bMoEtAPE was non-toxic even at high concentrations.

7.2 Synthesis and Characterization of Monomers and Polyesters

As previously reported, $N,N$-bis(hydroxyethyl) $N$-(alkyl)succinamide (HESA) monomers were used to synthesize TR-PEs that exhibited $T_{cp}$ up to 15.8 °C due to the overall hydrophobic-hydrophilic balance of the PE$s$. It was seen that TR-PEs containing tertiary amides exhibited a higher $T_{cp}$ than those based on secondary amides, a trend not always observed in poly(acrylamide)s (PAMs). Generally, amide hydrophilicity follows $3^\circ < 2^\circ < 1^\circ$. It is commonly accepted that increasing the overall polarity of a thermoresponsive polymer will increase the LCST. However, synthesizing monomers containing polar hydroxyl, amine, primary amide, and/or carboxylic acid functionality was decided against as the nucleophilic group would likely increase the degradation rate of the PE backbone.
Additionally, such a strategy would also require the use of protection-deprotection chemistry to prevent side reactions during the polymerization. Instead, the tertiary alkoxyamine bMoEtA, a structural analogue of diethylamine (dEtA, starting amine for TR-dEtAPE, \( T_{c,p} = 11.8 ^\circ C \)) bearing two polar methoxy groups, was chosen as a starting material. It was believed that the introduction of polar oxygen atoms would further increase the hydrophilic nature of the monomer, thereby shifting the hydrophobic/hydrophilic balance of the PE and increasing the overall LCST. bMoEtA was reacted with succinyl chloride to generate an \( N \)-alkoxy)succinamide ester intermediate (92%), which was then heated in the presence of diethanolamine (DEA) and purified via silica flash chromatography to yield bMo-EtADEA HESA monomer (80%). By undertaking this reaction under vacuum, displaced ethanol from the transamidation reaction was removed, driving the reaction forward and increasing the yield as compared to the previous TR-PE monomers.

First demonstrated by Stupp and coworkers,\textsuperscript{184} carbodiimide-mediated coupling has been shown to be an effective means of synthesizing high molecular weight PEs at room-temperature using a simple procedure (Scheme 7.1). In a typical polymerization, the bMoEtADEA diol and succinic acid (SA) were taken into anhydrous \( CH_2Cl_2 \). The reaction was cooled to 0 \( ^\circ C \) and DIC was added dropwise. The mixture was then allowed to stir for 24 – 48 h, with longer reaction times generally leading to higher molecular weight polymers. As the monomers, oligomers, polymer, DPTS catalyst, and DIC urea byproducts exhibited similar solubility in common solvents, purification by dialysis against MeOH was used to obtain pure polymers as tacky, low \( T_g \) elastomeric materials (65% polymer recovery). NMR confirmed purity of TR-bMoEtAPE (Figure 7.1).
Scheme 7.1: Synthetic route for the preparation of TR-bMoEtAPE. Reagents and conditions: (i) Et₃N, CH₂Cl₂, 0 °C to room-temperature, 1 h. (ii) DEA, neat, 80 °C, vacuum, 16 h. (iii) SA, DIC, DPTS, CH₂Cl₂, 0 °C to room-temperature, 48 h.

Figure 7.1: $^1$H NMR spectra of TR-bMoEtAPE (300 MHz, CDCl₃).

7.3 Thermoresponsive Behavior

Above the LCST, most thermoresponsive polymers undergo a relatively efficient dehydration and coil-globule transition, leading to a solid-liquid phase separation which is easily observed via DSC. However, certain polymers such as ELPs, poly(phosphoester)s (PPEs), and polar PAMs and poly(pyrrolidone)s exhibit an inefficient dehydration above their LCST.³⁵, ⁸⁶, ⁸⁹-⁹², ¹⁰⁴ The resulting liquid-liquid phase separation results in the formation of a polymer-rich coacervate phase and polymer-poor aqueous phase. Thermally
induced inefficient dehydration is often unobservable via DSC, so $T_{cp}$ is used to quantify the approximate LCST.

Using temperature controlled UV–vis, the sharp (occurring over ~3.5 °C) thermal response of TR-bMoEtAPE (132 kDa) was explored. As can be seen in Figure 7.2A, $T_{cp,\text{heat}}$ was observed at 48.2 °C and $T_{cp,\text{cool}}$ was observed at 39.6 °C. Hydrogen bond donating groups in thermoresponsive polymers have been shown to be a primary cause of thermal hysteresis (i.e., the difference between $T_{cp,\text{heat}}$ and $T_{cp,\text{cool}}$) as intra- and intermolecular hydrogen bonds form between the dehydrated polymer chains, making resolvation difficult.\textsuperscript{74} Although TR-bMoEtAPE contains only hydrogen bond accepting groups, a thermal hysteresis of 8.6 °C is observed. This value is greater than that of the previously reported TR-PEs and is likely due to the significant amount of water present in the semi-dehydrated coacervate phase. The water molecules likely form non-covalent intra- and intermolecular hydrogen bonded bridges between the many oxygen and nitrogen atoms present in polymer chains, making rehydration more difficult. A similar mechanism has been proposed for thermoresponsive poly(vinylpyrrolidone)s.\textsuperscript{213}
The effect of polymer concentration on $T_{cp}$ varies for different materials; typically, PAMs are not significantly affected by concentration, whereas poly(alkyloxazoline)s (PAOxs) and ELPs are much more dependent.\textsuperscript{14} Likewise, previous TR-PEs were shown to be highly dependent on solution polymer concentration.\textsuperscript{212} As the solution polymer concentration increases, more polymer is available in solution to form aggregates. This decreases the time required for dehydrated polymer globules to come together and form aggregates large enough to scatter light at the desired UV–vis wavelength. As expected, increasing TR-bMoEtAPE concentration resulted in an earlier onset of $T_{cp}$ ranging from 48.6 °C to 53.4 °C (Figure 7.2B). Above 1 wt %, the TR-bMoEtAPE concentration had little
affect on $T_{cp}$ and so 1 wt % was chosen as the standard concentration for UV–vis measurements.

7.5 Effect of Molecular Weight

Samples of TR-bMoEtAPE with varying molecular weights were prepared. As shown in Figure 7.2C, the $T_{cp}$ of TR-bMoEtAPE displays a logarithmic dependence on molecular weight, with low molecular weight samples exhibiting the highest $T_{cp}$ similar to what was observed to previous TR-PEs.\textsuperscript{212} In addition to the entropic differences of demixing experienced in systems of different molecular weight, contribution by the PE chain ends likely plays a role. The chain ends, likely polar hydroxyl or carboxylic acid groups, having a greater influence on the overall hydrophilicity of TR-bMoEtAPE resulting in an increased $T_{cp}$. Additionally, higher molecular weight TR-bMoEtAPEs likely exhibit increased hydrophobic polymer-polymer interactions, resulting in a decreased $T_{cp}$.

7.6 Thermal Cycle Testing

Thermal cycling showed reversible transitions between coacervate and water-soluble states with clear hysteresis between phase transitions. As seen in Figure 7.2D, the $T_{cp}$ remained relatively constant after several cycles. The $T_{cp,\ heat}$ increased from 48.2 °C to 49.0 °C, the $T_{cp,\ cool}$ increased from 39.6 °C to 41.1 °C, and the thermal hysteresis decreased from 8.6 °C to 7.9 °C. These data indicate that the temperature-induced phase transition of TR-bMoEtAPE is relatively stable and that the polymer does not undergo significant degradation, crosslinking, or removal from solution during the experimental timeframe.
7.7 Effect of Additives

It is well known that the Hofmeister salts affect the solubility of proteins and macromolecules in solution.\(^{214}\) Specifically, anions within the Hofmeister series are classified according to their ability to salt-out (kosmotropes, “disorder maker”) or salt-in (chaotropes, “order-maker”) proteins in a solution. Previous studies have shown that the LCST of thermoresponsive polymers can be modulated by Hofmeister ions: chaotropic salts increase the LCST, while kosmotropic salts decrease the LCST.\(^{23,215}\)

While previous theories to explain this behavior dealt with the effects of Hofmeister ions on bulk water properties, Cremer and coworkers put forth a possible mechanism for both PNIPAM and ELPs which involve the ions interacting directly with the polymers in three possible ways: destabilization of hydrogen bonding between water and polar groups through directed ionization of water by the anion (salting-out), an increased hydrophobic effect as salt is added to the solution (salting-out), and by direct binding of the anion to polar groups (salting-in).\(^{21,24}\) The effect of these interactions depends on ion size and charge density and generally follows the trend: \(\text{CO}_3^{2-} > \text{SO}_4^{2-} > \text{S}_2\text{O}_3^{2-} > \text{H}_2\text{PO}_4^{2-} > \text{F}^- > \text{Cl}^- > \text{Br}^- > \text{I}^- > \text{ClO}_4^- > \text{SCN}^-,\) with \(\text{CO}_3^{2-}\) acting as the strongest kosmotrope (lower LCST) and \(\text{SCN}^-\) acting as the strongest chaotrope (raise LCST).

To this end, the effects of Hofmeister sodium salts on TR-bMoEtAPE was explored. As shown in Figure 7.2D, the NaI, NaNO\(_3\), and NaBr were shown to have a chaotropnic effect, stabilizing the polymer in solution at elevated temperature. The chaotropic effect followed the expected relationship with Hofmeister anions, with NaI showing the greatest ability to salt-in TR-bMoEtAPE. Likewise, the kosmotropes NaCl, NaF, NaH\(_2\)PO\(_4\),
NaS$_2$O$_3$, and NaSO$_4$ were likewise shown to decrease the stability of hydrated TR-bMo-EtAPE chains in solution, resulting in a lowered $T_{cp}$ related to the kosmotropic strength of the anion. The only Hofmeister salt investigated in our previous studies, NaCl, was shown to have a greater effect on lowering $T_{cp}$ for TR-bMoEtAPE than was observed for earlier TR-PEs. This is likely due to the hydrogen bonds between water and the ether groups in TR-bMoEtAPE being more easily disrupted by NaCl than those between water and amides.$^{89}$ Interestingly, no $T_{cp}$ was observed for Na$_2$CO$_3$ and NaClO$_4$ at any concentration, likely due to rapid hydrolytic degradation of the PE in the basic solution.

The effect of urea on the thermoresponsive properties of TR-bMoEtAPE was also explored. Previously, the $T_{cp}$ of TR-PEs were shown to increase with increasing solution concentrations of urea, indicating that urea stabilized the solvated chains. A similar magnitude $T_{cp}$ increase is seen for TR-bMoEtAPE with increasing solution concentration of urea. The increase in $T_{cp}$ with urea for TR-PEs is comparable to that observed with ELPs, but opposite of the $T_{cp}$ depression reported for PNIPAM.$^{22}$ This is especially interesting since PNIPAM, being structurally similar to proteins and displaying temperature-dependent solubility, is often used as a model for investigating the cold denaturation of proteins and the effects that additives play in that process. Recently Feng and coworkers used urea-polymer Nuclear Overhauser Effect (NOE) measurements of poly($N$-isopropylacrylamide) (PNIPAM) and poly(diethylacrylamide) (PDEAM) to show that the type of amide as well as the size of hydrophobic domains play crucial roles in determining whether or not urea will stabilize the solvated chain as with PDEAM, increasing $T_{cp}$, or further dehydrate the polymer globule as with PNIPAM, decreasing $T_{cp}$. $^{73}$ Although similar factors likely
affect the stabilizing behavior urea demonstrates for TR-bMoEtAPE chains, the more complex structure of the polymer as compared to the PAMs makes direct comparison difficult. It is likely that the many predominantly hydrogen bond accepting groups (tertiary amides, ethers) of TR-bMoEtAPE results in solvating monovalent urea-polymer interactions as opposed to desolvating divalent interactions (i.e., ones where urea acts as a bridge between intra- and intermolecular hydrogen bonding sides). Our lab is currently exploring the use of UV–vis and NOE measurements to better understand urea-TR-PE interactions.

7.8 Copolymerization Results

It is well known that the LCST of a thermoresponsive polymer is effected by the overall hydrophobic-hydrophilic balance and as such can be tuned by copolymerization of monomers with differing hydrophilicity. This is especially true for random copolymers, even those that form coacervates.\textsuperscript{6,210} For polymers that do not interact, LCST tunability can be predicted using the formula:

\[ T = \mu_1 T_1 + \mu_2 T_2 \] (1)

where \( \mu \) is the mole fraction of each monomer and \( T \) is the \( T_{cp} \) of the corresponding homopolymer. As previously reported, the \( T_{cp} \) of TR-PE copolymers of similar molecular weight could be tuned in a linear fashion based on monomer feed from 7.8 °C to 15.9 °C based on monomer feed ratio. It was hypothesized that a similar effect would be seen for copolymers containing bMoEtA and iPrA pendant groups of similar molecular weight. As shown in
Figure 7.3, (dashed black line), increasing the content of more hydrophilic bMoEtA increased the overall hydrophilicity of the polymer, thereby increasing the $T_{cp}$ with good agreement to the predicted values. Interestingly, the $T_g$ of the copolyesters was seen to be inversely correlated to the cloud point temperature and decreased with bMoEtA content and appears to follow the Fox equation:

$$\frac{1}{T_g} = \frac{w_1}{T_{g,1}} + \frac{w_2}{T_{g,2}}$$  \hspace{1cm} (2)

where $w$ is the weight fractions of each polymer (in this case, the mole fraction of each monomer in the copolymer) and $T_g$ is the glass transition temperature of the corresponding homopolymer (Table 7.1, Figure 7.3, dashed blue line)

Table 7.1: Characterization of TR-(bMoEtA-r-iPrA)PE Copolyesters

<table>
<thead>
<tr>
<th>bMoEtA : iPrA</th>
<th>$M_a$</th>
<th>$T_{cp, exp}$</th>
<th>$T_{cp, theo}$</th>
<th>$T_{g, exp}$</th>
<th>$T_{g, theo}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 : 0</td>
<td>45.6</td>
<td>53.4</td>
<td>53.4</td>
<td>-7.51</td>
<td>-7.51</td>
</tr>
<tr>
<td>0.75 : 0.25</td>
<td>59.1</td>
<td>39.8</td>
<td>41.9</td>
<td>-3.85</td>
<td>-3.34</td>
</tr>
<tr>
<td>0.50 : 0.50</td>
<td>61.8</td>
<td>27.2</td>
<td>30.4</td>
<td>-0.45</td>
<td>0.97</td>
</tr>
<tr>
<td>0.25 : 0.75</td>
<td>61.1</td>
<td>13.3</td>
<td>18.8</td>
<td>7.31</td>
<td>5.41</td>
</tr>
<tr>
<td>0 : 1.0</td>
<td>56.5</td>
<td>7.3</td>
<td>7.3</td>
<td>10.0</td>
<td>10.00</td>
</tr>
</tbody>
</table>

aDetermined by DMF SEC relative to PMMA standards. bDefined as 50% transmission during temperature controlled UV–vis analysis. cDetermined by DSC.
Figure 7.3: Effect of copolyester composition on $T_{cp}$ and $T_g$ for a series of TR-(bMoEtA-$r$-iPrA)PE of various $M_n$. Dashed lines represents theoretical values using the Fox equation (blue) and a weighted average (black).

The correlation is likely due to a number of factors. A decrease in hydrogen bond donating secondary iPrA amides decreases polymer-polymer interactions resulting in a reduction of $T_g$. Additionally, the $T_g$ is likely lowered with increasing the amount of flexible bMoEtA methoxy branches as the polymer free volume increases and disrupts packing. These data represent a significant increase in overall thermal and physical tunability for the TR-PE system, as the range of $T_{cp}$ could be tuned theoretically in the range of 0 – 55 °C. Furthermore, the $T_g$ of the TR-PEs eliminates the possibility of forming glassy material above the LCST, a drawback for many thermoresponse polymers such as PNIPAM ($T_g \sim 140 ^\circ C$).
7.9 Coacervate Analysis

It was predicted that TR-bMoEtAPE would form polymer-rich coacervates above the LCST due to their structural similarities to previous TR-PEs. Centrifugation of TR-bMoEtAPE solutions above the $T_{cp}$ resulted in a dense, transparent liquid phase, not a precipitate, indicating a coacervation-type dehydration. Variable temperature $^1$H NMR was used to further verify the coacervation response. As seen in Figure 7.4A, peaks corresponding to the backbone and pendant protons are seen to decrease with increasing temperature. The disappearance of an individual signal typically occurs when local dehydration occurs, resulting in a lack of segmental mobility and can give insight as to the mechanism of temperature-induced phase transition.\(^8^3\) However, even above the $T_{cp}$, no proton signals completely disappeared. This suggested that the polymer was still flexible and well hydrated above the LCST, indicative of coacervate-type demixing. From the normalized proton integral signals (Figure 7.4B), the mechanism of dehydration was partially explored. Between 35 – 40 ºC, the signals corresponding to the pendant OCH$_3$ and backbone CH$_2$O protons decreased to ~85% of their initial value. In comparison, the signals corresponding to the protons adjacent to the amide and carbonyl groups exhibited a weaker reduction in signal (~92 – 95%), indicating less disruption of the hydrogen bonding between those protons and D$_2$O. Between 40 – 45 ºC, all four signals remained in the range of 82 – 87% before rapidly decreasing when heated above the $T_{cp}$ to final non-zero values ranging from 53 – 61%. Taken together, these data suggest that while the pendant OCH$_3$ and backbone CH$_2$O moieties contain the weakest polymer-D$_2$O hydrogen bonding, above the LCST the
various domains of TR-bMoEtAPE dehydrate in a cooperative manner leading to coacervation. Cooperative dehydration above the LCST is further reinforced by the sharp $T_{cp}$ transition range (~3.5 ºC).

Figure 7.4: (A) Variable temperature $^1$H NMR spectra of TR-bMoEtAPE (400 MHz, D$_2$O, $T_{cp} = 48.1$ ºC) above and below cloud point and (B) the normalized proton integral signals.

The polymer concentration of the TR-bMoEtAPE coacervates was determined to be 36.3%. This is lower than the previously investigated TR-PEs (44.2 – 65.4%), where it was observed that $T_{cp}$ was inversely correlated to the coacervate concentration of polymer. Above the LCST, the more hydrophilic TR-bMoEtAPE likely undergoes an even less efficient dehydration than previous TR-PEs, resulting in increased coacervate water content and decreased polymer content coacervate.

The sizes of TR-bMoEtAPE coacervates at different temperatures was examined using DLS. A lower molecular weight TR-bMoEtAPE (45.6 kDa, $T_{cp} = 53.5$ ºC) was used in order to eliminate any molecular weight effect when making comparisons to previous TR-PEs. Generally, previous TR-PE coacervate size (102 – 226 nm) was seen to increase with $T_{cp}$ and coacervate water content. As shown in Figure 7.5, below LCST the polymers exist primarily as solvated unimers with $R_{h, app} = ~ 8$ nm. As the solution is heated above
the LCST, the $R_{h, \text{app}}$ increases to 231 nm at 54 °C as initial coacervates form. The observed LCST from DLS matches relatively well with the $T_{cp}$. Interestingly, the more hydrophilic TR-bMoEtAPE coacervates are only slightly larger than the TR-PyrAPE (56.5 kDa, $T_{cp} = 15.8$ °C, 226 nm, polymer coacervate concentration = 44.2%) coacervates despite the increased coacervate water content.

Figure 7.5: CONTIN analysis of the DLS data of TR-bMoEtAPE ($T_{cp} = 53.5$ °C) above and below the cloud point.

7.10 Polymer Degradation

PE-type materials are inherently degradable in biological systems due to the hydrolysis of the ester linkages by water. The rate of hydrolysis is dependent on a number of factors. For instance, the rate of poly(lactic acid) (PLA) degradation can vary from months to years depending on pH, tacticity, crystallinity, temperature, and polymer hydrophobicity.$^{131, 216}$ However, there is a need for more rapidly degrading systems, especially within the field of targeted drug delivery.
Previous work in our lab has shown that PEs based on SA and N-substituted diols degrade relatively quickly. At 37 °C, the “peptide-like” polyester p(mAla) degraded to 66% of the starting $M_n$ after 7 days,\(^9\) while the more hydrophilic TR-PE polyester TR-dEtPE degraded to 30% of the original $M_n$.\(^{212}\) As shown in Figure 7.6, TR-bMoEtAPE was observed to degrade from 132 kDa to an average of 38.7 kDa, or 29.3% of the original $M_n$, over a period of 7 days in DI water. The magnitude of degradation was similar to that of other TR-PEs.

Since it has been shown that lower molecular weight TR-PEs have higher $T_{cp}$, a solution of TR-bMoEtAPE in PBS buffer was incubated at 37 °C to explore degradation under biological conditions. Over a period of 7 days, a clear shift in $T_{cp}$ from 44.2 °C to 74.5 °C was observed, with the transmittance curve at longer degradation times beginning to show a two-step transmittance transition. As the $T_{cp}$ of TR-PEs exhibit a significant dependence on both concentration and molecular weight below 20 kDa, the observed change in transmittance suggests the presence of highly variable molecular weight degradation products whose temperature-induced dehydration are independent of one another.\(^{217}\) The multi-step transmittance likely occurs as follows: below the LCST, all TR-bMoEtAPE chains are soluble. As the temperature is raised, the higher molecular weight chains begin to dehydrate first. The hydrophobic dehydrated chains begin to aggregate only with other high molecular weight dehydrated chains, not the hydrated lower molecular weight chains. The aggregated high molecular weight dehydrated chains begin to scatter light and cause initial drop in transmittance. The magnitude of the transmittance drop is indicative of both the molecular weight of the dehydrated chains and total number of chains of that molecular weight. The second drop comes as the more soluble lower molecular weight chains are
brought above their LCST, resulting in a drop to near-zero transmittance as nearly all chains in solution, regardless of molecular weight, are desolvated, aggregated, and scattering light.

![Graph](image1.png)

Figure 7.6: Left: Hydrolytic degradation via SEC of TR-bMoEtAPE incubated 37 °C in DI water; n = 3. Right: hydrolytic degradation via UV–vis of TR-bMoEtAPE incubated at 37 °C in 1X PBS buffer.

7.11 Cell Viability

Since TR-bMoEtAPE was designed for possible biomedical applications, cell viability studies were used to probe possible toxicity. TR-bMoEtAPE was incorporated into cell growth medium at various concentrations and added to proliferating NIH 3T3 mouse embryonic fibroblast cells. After 1 day of growth, cell viability was probed. As shown in Figure 7.7, even at high concentrations (1 mg/mL) of TR-bMoEAPE, the cell viability remained relatively consistent with the non-inoculated control sample. This suggests that TR-bMoEtAPE is relatively non-cytotoxic and may show promise for biomedical applications.
Figure 7.7: Cell viability of TR-bMoEtAPE against NIH 3T3 cells, 1 day; n = 3.

7.12 Conclusions

In this work, we have expanded the previously described TR-PE system through the synthesis of a hydrophilic monomer based on bMoEtA. The resultant PE, TR-bMoEtAPE, displayed a sharp and highly reversible $T_{cp}$ around 50 ºC, a three-fold increase as compared to previous TR-PEs. Similar fast hydrolytic degradation and coacervation-type dehydration were observed as compared to previous TR-PEs. Variable temperature NMR indicated temperature-induced dehydration is a cooperative event. Moreover, the thermal and physical properties were highly tunable and predictable via copolymerization with previous TR-PE monomers. Preliminary cell viability experiments suggested TR-bMoEtAPE is non-cytotoxic even at high concentrations. The increased LCST and tunability makes TR-bMoEtAPE an ideal candidate for future copolymerization studies with functionalized diol monomers.
CHAPTER VIII

EFFECT OF STRUCTURE ON THERMAL AND THERMORESPONSIVE PROPERTIES OF N-SUBSTITUTED POLYESTERS

8.1 Introduction

In the previous two chapters, a new system of high molecular weight, biodegradable, thermoresponsive polyesters (TR-PEs) showing Lower Critical Solution Temperature (LCST) behavior has been discussed. In Chapter VI, the initial design and testing of the TR-PE system inspired by poly(acrylamide)s (PAMs) was explored. In a two-step synthetic route, a series of \(N,N\)bis(hydroxyethyl) \(N\)-(alkyl)succinamide (HESA) monomers were polymerized to generate polyesters which underwent inefficient temperature-induced phase separation leading to coacervation at temperatures ranging from 0 – 15.8 ºC. The TR-PEs were shown to undergo relatively quick degradation and were capable of encapsulating hydrophobic model compounds. In Chapter VII, the system was expanded by designing an even more hydrophilic \(N\)-(alkoxy)succinamide HESA monomer inspired by poly(alkoxyacrylamide)s (PAOAMs). The increased TR-PE hydrophilicity resulted in a threefold increase in the cloud point temperature (\(T_{cp}\), an approximation of the LCST) that could be easily tuned via copolymerization with less hydrophilic monomers. Moreover, preliminary studies indicated the materials to be non-cytotoxic even at high concentrations.
Since TR-PEs are structurally more complex than other thermoresponsive materials, it can be difficult to predict how the amide substituents will directly affect the thermal and physical properties of the material. However, by using the synthetic route outlined in the previous chapters it follows that the number of unique HESA monomers (and in turn, TR-PEs) is limited only by the availability of commercially or synthetically available primary and secondary amines. Building a more diverse TR-PE library would aid not only in the understanding and prediction of material properties, but one can imagine a number of instances where a more diverse library of TR-PE diol monomers would be desirable. For instance, monomers bearing bulky or less flexible pendant groups could aid in tuning the glass transition temperature ($T_g$) of TR-PEs via copolymerization (which has been already shown to follow the Fox equation). Increasing monomer hydrophilicity would widen the range of hydrophobic monomers that could be copolymerized to generate a thermoresponsive material. Likewise, hydrophobic monomers could be used to counterbalance the incorporation of highly hydrophilic charged monomers. Furthermore, based on the significant increase in LCST afforded to TR-PEs by inclusion of alkoxy substituents, it may be possible to synthesize analogous $N,N$-bis(hydroxyethyl) $N$-(alkyl or alkoxy)amide (HEA) monomers in a simplified one step reaction to create TR-PEs.

This chapter describes the synthesis and characterization of 20 PEs, including the six previously reported TR-PEs. The effect of $N$-substituted pendant groups on physical, thermal, and thermoresponsive properties of the PEs was probed. Finally, the covalent attachment of a model drug to create a thermoresponsive, biodegradable therapeutic carrier was explored.
8.2 Synthesis of Monomers and Polymers

Scheme 8.1: Synthetic route for the preparation of TR-bMoEtAPE. Reagents and conditions: (i) Et$_3$N, CH$_2$Cl$_2$, 0 °C to room-temperature, 1 h. (ii) DEA, neat, 80 °C, vacuum, 16 h. (iii) Et$_3$N, MeOH, 60 °C microwave, 2 h. (iv) Ibuprofen, DIC, DPTS, CH$_2$Cl$_2$, 0 °C to room-temperature, 16 h. (v) DEA, neat, 70 – 80 °C microwave, 30 min. (vi) SA, DIC, DPTS, CH$_2$Cl$_2$, 0 °C to room-temperature, 48 h.

The synthesis of monomers followed the same general procedure described in previous chapters (Scheme 8.1). For HESA monomers, a primary or secondary amine was reacted with ethyl succinyl chloride. After stirring for 1 h, the solution is purified via an aqueous workup to generate a succinamide ester intermediate in quantitative yields. The ester intermediates were heated in the presence of diethanolamine (DEA) under vacuum and purified via silica flash chromatography to yield the desired HESA monomer (50-80%). For HEA monomers, an alkyl/alkoxy ester is heated in the presence of diethanolamine (DEA) and purified via silica flash chromatography to yield the desired HEA monomer (60-70%). Microwave heating was not used for HESA monomer synthesis as many of the succinamide esters were seen to undergo cyclization and proved difficult to purify.

The ibuprofen (ibu) containing monomer (ibuDEA) was synthesized in a multistep reaction. First, γ-butyrolactone was ring opened by methanol (MeOH) in a microwave reactor. The resultant pale oil was determined to be 75% pure hydroxyester via NMR and
was used without further purification. The hydroxyester was coupled to ibuprofen via DPTS catalyzed carbodiimide coupling. After silica flash chromatography, the pure ibuprofen-ester intermediate was obtained in quantitative yields. In order to preferentially transamidate the methylester with DEA and reduce unwanted transamidation of the sterically hindered ibuprofen-ester, coupling of the ibuprofen-ester intermediate to DEA was undertaken via microwave conditions. The concentrated oil was purified via silica flash chromatography to yield pure ibuDEA monomer (50%) as a pale oil.

In a typical polymerization, the desired diol monomer and succinic acid (SA) were taken into anhydrous CH$_2$Cl$_2$. The reaction was cooled to 0 °C and DIC was added dropwise. The mixture was then allowed to stir for 24 – 48 h, with longer reaction times generally leading to higher molecular weight polymers. After reaction completion, the solution was diluted with CH$_2$Cl$_2$ and urea was filtered off. TR-PEs based on HESA monomers were purified by dialysis against MeOH while TR-PEs based on HEA monomers were purified by precipitation into cold MeOH. The purified TR-PEs were observed to be tacky, low $T_g$ elastomeric materials (Figure 8.1). Purity was confirmed via NMR.
8.3 Characterization of $N$-amide Polyesters

A significant advantage of the TR-PE system is the modular synthetic route which facilitates a variety of homo- and copolymers that are not readily achievable with traditional ROP. There exist many structural differences between TR-PEs and other thermoreponsive polymers as well as PEs in general: backbone flexibility, functional group density, hydrophobic/hydrophilic balance, and hydrogen bond sides. However, these factors likely influence the thermal and physical properties of the TR-PEs in a similar manner to what is observed for other polymers.

In the previous chapter, it was noted that the TR-PE with the highest $T_{cp}$, TR-bMo-EtAPE (TR13, $T_{cp} \sim 50 ^\circ C$, $T_g \sim -15 ^\circ C$) displayed a glass transition temperature ($T_g$) much lower than that of the TR-PE with the lowest observable $T_{cp}$, TR-iPrAPE (TR7, $T_{cp} \sim 7 ^\circ C$, $T_g \sim 10 ^\circ C$). It was hypothesized that there might be a correlation between temperature
dependent solubility and TR-PEs physical properties, such as $T_g$. However, the TR-PE library was not yet large enough to allow for general trends to be seen and predicted. To this end, we expanded the TR-PE library to include substituents based on a variety of alkyl and alkoxy substituted amides (TR1 – 14), as well as alkyl and alkoxy groups (TR15 – 19, to be discussed in further detail below). The physical and thermal properties of the amide TR-PEs (Table 8.1) were observed to be influenced by the type of amide (secondary vs. tertiary), the type of amide substituent (alkyl vs. alkoxy), as well as the structure of the amide substituent (size, and linear vs. ring).

The thermal degradation temperature ($T_{\text{deg}}$) of all amide TR-PEs remained in the range of ~270 – 315 °C. However, a comparative analysis of secondary and tertiary amide based TR-PEs reveals that in general, TR-PEs bearing tertiary amide substituents were more thermally stable by ~30 – 40 °C. This suggests that the hydrogen-containing secondary amide functional groups are less thermally stable than their fully substituted tertiary counterparts. Likewise, the propargyl amide is an outlier with the lowest thermal stability (TR3, 242 °C), likely a result of the low molecular weight as well as the unstable alkyne functionality.

The $T_g$ of a polymer is an important physical property for medical applications and can be affected by a number of factors. First, it should be noted that the variation in $T_g$ for amide TR-PEs is not expansive, likely as a result of the common flexible backbone and low functional group density. Still, a number of correlations can be drawn. The most important factor appears to be hydrogen bonding. Generally, TR-PEs bearing amide protons are able to form hydrogen bonds with the numerous oxygen and nitrogen atoms in the TR-
PE structure. These intra- and intermolecular bonds result in reduced polymer mobility and an increased $T_g$ (6 – 13 ºC) as compared to the tertiary amide-based TR-PEs with linear substituents (-20 – 0 ºC). Within the class of amide TR-PEs, more generalizations could be made with regards to $T_g$. For tertiary amides, the presence of large rigid rings (TR4) greatly increased the $T_g$ as compared to smaller rings (TR9), but the difference was reduced when a flexible oxygen atom was incorporated (TR12). The slightly less bulky $i$-propyl

Table 8.1: Characterization of amide TR-PEs.

<table>
<thead>
<tr>
<th>Polyester</th>
<th>Label</th>
<th>$M_n^a$ (kDa)</th>
<th>$M_w^a$ (kDa)</th>
<th>$D_M$</th>
<th>$T_g^b$ (ºC)</th>
<th>$T_{deg}^c$ (ºC)</th>
<th>Structural formula of amide</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-cPrAPE</td>
<td>TR1</td>
<td>25.5</td>
<td>42.4</td>
<td>1.6</td>
<td>14</td>
<td>273</td>
<td>NHCH(CH$_2$)$_2$</td>
</tr>
<tr>
<td>TR-nPrAPE</td>
<td>TR2</td>
<td>29.1</td>
<td>43.9</td>
<td>1.5</td>
<td>5</td>
<td>271</td>
<td>NHCH$_2$CH$_2$CH$_3$</td>
</tr>
<tr>
<td>TR-PropargylAPE</td>
<td>TR3</td>
<td>12.9</td>
<td>17.2</td>
<td>1.3</td>
<td>13</td>
<td>242</td>
<td>NHCH$_2$CCH$_3$</td>
</tr>
<tr>
<td>TR-PPDAPE</td>
<td>TR4</td>
<td>26.0</td>
<td>36.5</td>
<td>1.4</td>
<td>22</td>
<td>309</td>
<td>N(CH$_3$)$_3$</td>
</tr>
<tr>
<td>TR-dBuAPE</td>
<td>TR5</td>
<td>44.5</td>
<td>63.0</td>
<td>1.4</td>
<td>-4</td>
<td>315</td>
<td>N(CH$_2$CH$_2$CH$_3$)$_2$</td>
</tr>
<tr>
<td>TR-HepAPE</td>
<td>TR6</td>
<td>56.8</td>
<td>76.1</td>
<td>1.3</td>
<td>9</td>
<td>277</td>
<td>NH(CH$_2$)$_6$CH$_3$</td>
</tr>
<tr>
<td>TR-iPrAPE</td>
<td>TR7</td>
<td>56.5</td>
<td>88.5</td>
<td>1.6</td>
<td>10</td>
<td>287</td>
<td>NHCH(CH$_3$)$_2$</td>
</tr>
<tr>
<td>TR-dEtAPE</td>
<td>TR8</td>
<td>72.7</td>
<td>112.2</td>
<td>1.4</td>
<td>-4</td>
<td>304</td>
<td>N(CH$_2$CH$_3$)$_2$</td>
</tr>
<tr>
<td>TR-PyrAPE</td>
<td>TR9</td>
<td>54.2</td>
<td>86.6</td>
<td>1.6</td>
<td>0</td>
<td>307</td>
<td>N(CH$_2$)$_4$</td>
</tr>
<tr>
<td>TR-THFAPE</td>
<td>TR10</td>
<td>61.1</td>
<td>85.6</td>
<td>1.4</td>
<td>13</td>
<td>275</td>
<td>NHCH$_2$CHCH$_2$CH$_2$CH$_3$O</td>
</tr>
<tr>
<td>TR-EoEtAPE</td>
<td>TR11</td>
<td>40.8</td>
<td>60.7</td>
<td>1.5</td>
<td>9</td>
<td>273</td>
<td>NHCH$_2$CH$_2$OCH$_2$CH$_3$</td>
</tr>
<tr>
<td>TR-MorAPE</td>
<td>TR12</td>
<td>31.4</td>
<td>42.4</td>
<td>1.4</td>
<td>4</td>
<td>306</td>
<td>N(CH$_2$CH$_2$OCH$_2$CH$_2$)</td>
</tr>
<tr>
<td>TR-bMoEtAPE</td>
<td>TR13</td>
<td>133.5</td>
<td>211.4</td>
<td>1.6</td>
<td>-20</td>
<td>315</td>
<td>N(CH$_2$CH$_2$OCH$_3$)$_2$</td>
</tr>
<tr>
<td>TR-MoEtAPE</td>
<td>TR14</td>
<td>43.7</td>
<td>58.9</td>
<td>1.4</td>
<td>6</td>
<td>266</td>
<td>NHCH$_2$CH$_2$OCH$_3$</td>
</tr>
</tbody>
</table>

$^a$Determined by DMF SEC relative to PMMA standards. $^b$Determined by DSC. $^c$Determined by TGA
substituent (TR7) inhibits packing less than its \( c \)-propyl counterpart (TR1), which could explain its slightly lower \( T_g \). Amide TR-PEs containing long linear substituents (TR5, 8, and 13) are more likely to disrupt polymer packing and thus displayed some of the lowest \( T_g \) values. Furthermore, the large number of oxygen atoms present in the alkoxy substituents of TR13 likely reduce packing to an even greater degree, resulting in the lowest \( T_g \) for any TR-PE.

The solution properties of amide TR-PEs are much simpler to predict and explain. It is intuitive that TR1 – 6 would not display a LCST-type behavior given the hydrophobicity of the alkyl amide substituents. In line with earlier observations, it was noted that in order to generate TR-PEs with increased LCST, the starting monomers would have to be made more hydrophilic. This could be achieved using two strategies: reduction of hydrophobic methylene groups in the amide substituents, or addition of hydrophilic heteroatoms. The first strategy was decided against for safety reasons as it would have required the handling of highly dangerous methyl- or dimethylamine gas. To this end, four different alkoxyamines were chosen as starting materials based on the thermoresponsive behavior of the corresponding PAOAMs\(^{48}\) and commercial availability. The temperature dependent phase transition behavior of the TR-PEs in DI water and PBS buffer is shown in Figure 8.2.

The amide TR-PEs based on tetrahydrofurfurylamine (TR-THFAPE, TR10) and ethoxyethylamine (TR-EoEtAPE, TR11) showed sharp \( T_{cp} \) values around 19 \( ^\circC \) and 35 \( ^\circC \), respectively, whereas the literature \( T_{cp} \) values of the corresponding PAOAMs are between 33 – 38 \( ^\circC \).\(^{47-49,81}\) The magnitude of \( T_{cp} \) difference observed with TR10 (~16 \( ^\circC \)) and TR11 (~2 \( ^\circC \)) as compared to the model PAOAM is due to the difference in the overall hydrophobic/hydrophilic balance of the TR-PEs and their models. It should be noted that the
magnitude of the decrease is much less than what is observed with the alkyl PAM mimic polymers TR7 – 9, which display $T_{cp}$ between $\sim$20 – 35 °C less than expected by their PAM models. It is believed that the incorporation of oxygen heteroatoms plays a much greater role in modulating the LCST behavior of the TR-PEs than simply relying on adjustment of methylene groups. The reason why the $T_{cp}$ of TR11 is nearly identical to the corresponding PAOAM is still not well understood. Additionally, two TR-PEs based on morpholine (TR-MorAPE, TR12) and methoxyethylamine (TR-MoEtAPE, TR14) were synthesized, as the corresponding PAOAMs were known to be fully water soluble.\cite{40,48,218}

Table 8.2: Solution properties of amide TR-PEs.

<table>
<thead>
<tr>
<th>Polyester</th>
<th>Label</th>
<th>$M_n^a$ (kDa)</th>
<th>$T_g^b$ (°C)</th>
<th>$T_{cp, \text{heat}}^c$ (°C)</th>
<th>$T_{cp, \text{cool}}^c$ (°C)</th>
<th>Hyst.$^c$ (°C)</th>
<th>95-5%$T^c$ (°C)</th>
<th>C (#)</th>
<th>O (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-cPrAPE</td>
<td>TR1</td>
<td>25.5</td>
<td>14</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TR-nPrAPE</td>
<td>TR2</td>
<td>29.1</td>
<td>5</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TR-PropargylAPE</td>
<td>TR3</td>
<td>12.9</td>
<td>13</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TR-PPDAPE</td>
<td>TR4</td>
<td>26.0</td>
<td>22</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>TR-dBuAPE</td>
<td>TR5</td>
<td>44.5</td>
<td>-4</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>TR-HepAPE</td>
<td>TR6</td>
<td>56.8</td>
<td>9</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>TR-iPrAPE</td>
<td>TR7</td>
<td>56.5</td>
<td>10</td>
<td>7(3)</td>
<td>N/A</td>
<td>N/A</td>
<td>3(3)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TR-dEtAPE</td>
<td>TR8</td>
<td>72.7</td>
<td>-4</td>
<td>12(11)</td>
<td>7(2)</td>
<td>5(8)</td>
<td>3(3)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>TR-PyrAPE</td>
<td>TR9</td>
<td>54.2</td>
<td>0</td>
<td>16(13)</td>
<td>9(6)</td>
<td>7(7)</td>
<td>4(4)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>TR-THFAPE</td>
<td>TR10</td>
<td>61.1</td>
<td>13</td>
<td>19(17)</td>
<td>11(9)</td>
<td>8(8)</td>
<td>4(3)</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>TR-EtEtAPE</td>
<td>TR11</td>
<td>40.8</td>
<td>9</td>
<td>35(31)</td>
<td>29(23)</td>
<td>6(8)</td>
<td>5(4)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>TR-MorAPE</td>
<td>TR12</td>
<td>31.4</td>
<td>4</td>
<td>40(36)</td>
<td>36(36)</td>
<td>4(0)</td>
<td>17(9)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>TR-bMoEtAPE</td>
<td>TR13</td>
<td>133.5</td>
<td>-20</td>
<td>48(44)</td>
<td>40(37)</td>
<td>9(7)</td>
<td>4(3)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>TR-MoEtAPE</td>
<td>TR14</td>
<td>43.7</td>
<td>6</td>
<td>77(67)</td>
<td>85(*)</td>
<td>-8(*)</td>
<td>29(*)</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\)Determined by DMF SEC relative to PMMA standards. \(^b\)Determined by DSC. \(^c\)Determined by UV–vis for PE solutions in DI H$_2$O (values in parentheses are for 1X PBS). \(*\) Not observed.
Interesting, TR12 displayed a broad phase transition (i.e., the temperature range required to bring the transmittance from 95% to 5%) with the $T_{cp}$ generally occurring at 40 ºC. TR-PEs of TR14 were fully water soluble at molecular weights below 35 kDa. Only at 43.7 kDa was a broad phase transition was observed with a calculated $T_{cp}$ of 77 ºC. This interesting observation is likely due to the more polar secondary amide, as well as the ratio of oxygen to carbon in the substituent being quite high, and makes the TR14 monomer an ideal candidate for copolymerization with hydrophobic monomers. The broad phase transition ranges of TR12 and TR14 are not likely a consequence of polydispersity, which is similar for all TR-PEs, but are instead suggestive of a less cooperative dehydration above the LCST than is observed with other TR-PEs. It is likely that the less hindered alkoxy groups are able to better maintain their hydrogen bonds to water. The mechanism of dehydration is currently under investigation using temperature controlled IR and NMR studies.

From these data, a few generalizations among the solution behavior of all amide TR-PEs could be made. First, all amide TR-PEs were shown to be sensitive to the addition of cosalt, as the $T_{cp}$ observed in DI water is typically 2 – 5 ºC higher than in PBS solution.
Second, all TR-PES displayed a solution hysteresis (i.e. the difference between $T_{cp, \text{heat}}$ and $T_{cp, \text{cool}}$) as the inefficient dehydration above LCST results in the formation of a coacervate phase with many water molecules acting as hydrogen bonded bridging points between the heteroatoms of the TR-PE. This results in a slower rehydration of the PE chains when brought below the LCST, as replacing the bridging water-polymer hydrogen bonds with those of bulk water takes longer to reach equilibrium than dehydrated chains held together mainly through polymer-polymer hydrophobic interactions. The magnitude of hysteresis did not seem to be affected by the type of amide, suggesting that the amide hydrogen does not play a significant role in intra- and intermolecular hydrogen bonding in the coacervate phase. In line with previous observations, the $T_{cp}$ of all TR-PES exhibited a logarithmic dependence on molecular weight, with lower molecular weights having higher $T_{cp}$ values (Figure 8.3). Overall the $T_{cp}$ could not be predicted from the $T_g$ of the amide TR-PE as seen in Figure 8.4A. There did seem to be a trend of $T_{cp}$ with the number of oxygen and carbon atoms present in the amide substituent. For amide TR-PES with greater than four carbons, no $T_{cp}$ is seen unless oxygen atoms are included in the substituent (Figure 8.4B). Interestingly, the $T_{cp}$ as a function of the ratio oxygen to carbon atoms in the amide substituent does seem to show a linear trend, regardless of amide type or copolymerization (Figure 8.4C). It appears that TR-PES bearing amide substituents lacking any oxygen atoms are limit to an upper $T_{cp}$ limit of ~16 °C. Raising the O/C ratio to 1 : 4 appears best for generating amide TR-PE homopolymers exhibiting a $T_{cp}$ near that of body temperature, while further increasing that range to 1 : 3 increases the overall temperature from 50 °C to
Figure 8.3: The effect of molecular weight on $T_{cp}$ for TR-PEs. (10 mg/mL, 1 ºC/min heating rate, DI water). Trend lines are added as a visual aid. TR-MoEtAPE is soluble below $M_n \sim 35$ kDa.

Figure 8.4: The $T_{cp}$ of amide TR-PEs as a function of $T_g$ (A), carbon and oxygen number of amide substituents (B), and O/C ratio of amide substituents (C).
completely soluble. Taken together, these data bolster the tunability of the TR-PE system and give insight to the design of future thermoresponsive PEs as will be discussed in further detail below.

8.4 Characterization of $N$-alkyl & alkoxy Polyesters

Previously, the first attempt to synthesize TR-PEs relied on $N$-alkyl substituted PEs inspired by thermoresponsive poly(alkyl oxazoline)s (PAOxs). It was hypothesized that the relatively hydrophilic backbone of the PE would be balanced out by the short hydrophobic $N$-alkyl chain. However, the $n$-propyl (TR15), and $i$-propyl (TR16) and ethyl (TR17) polymers were not observed to be water soluble. As such, $N$-methyl PE (TR-18) was synthesized but was also found to be insoluble in cold water. Polyesterification of methylidioethanolamine was attempted, but the reaction turned brown and no polymeric material was recovered, likely as a result of the basic monomer affecting the DPTS acid-base equilibria. However, it can be seen that as the $N$-alkyl substituent was made longer, PE chain packing is inhibited resulting in a reduced $T_g$ (Table 8.3).

Since the incorporation of polar alkoxy substituents was seen to raise the $T_{cp}$ of amide-based TR-PEs, we hypothesized that a similar trend would be observed for $N$-alkoxy PEs. To this end, we synthesized PEs bearing ethoxymethyl (TR-EoMePE, TR19) and methoxymethyl (TR-MoMePE, TR20) $N$-substituents. The $T_{deg}$ and $T_g$ were similar to the closest alkyl analogue, TR14, and were in line with the previously discussed amide TR-PEs (Table 8.3).
Table 8.3: Characterization of alkyl & alkoxy TR-PEs.

<table>
<thead>
<tr>
<th>Polyester</th>
<th>Label</th>
<th>(M_n^a) (kDa)</th>
<th>(M_w^a) (kDa)</th>
<th>(D_M)</th>
<th>(T_g^b) (ºC)</th>
<th>(T_{deg}^c) (ºC)</th>
<th>Structural formula of side chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>nPrPE</td>
<td>TR15</td>
<td>25.3</td>
<td>39.8</td>
<td>1.57</td>
<td>-20</td>
<td>**</td>
<td>CH₂CH₂CH₃</td>
</tr>
<tr>
<td>iPrPE</td>
<td>TR16</td>
<td>17.1</td>
<td>23.5</td>
<td>1.34</td>
<td>-2</td>
<td>**</td>
<td>CH(CH₃)₂</td>
</tr>
<tr>
<td>EtPE</td>
<td>TR17</td>
<td>58.0</td>
<td>75.4</td>
<td>1.30</td>
<td>6</td>
<td>282</td>
<td>CH₂CH₃</td>
</tr>
<tr>
<td>MePE</td>
<td>TR18</td>
<td>32.2</td>
<td>47.1</td>
<td>1.46</td>
<td>-5</td>
<td>**</td>
<td>CH₃</td>
</tr>
<tr>
<td>TR-EoMePE</td>
<td>TR19</td>
<td>75.4</td>
<td>110.7</td>
<td>1.47</td>
<td>-13</td>
<td>294</td>
<td>CH₂OCH₂CH₃</td>
</tr>
<tr>
<td>TR-MoMePE</td>
<td>TR20</td>
<td>32.8</td>
<td>43.1</td>
<td>1.31</td>
<td>-9</td>
<td>290</td>
<td>CH₂OCH₃</td>
</tr>
</tbody>
</table>

\(^a\)Determined by DMF SEC relative to PMMA standards. \(^b\)Determined by DSC. \(^c\)Determined by TGA. **Data not available at the time of publication.

Table 8.4: Solution properties of alkyl & alkoxy TR-PEs.

<table>
<thead>
<tr>
<th>Polyester</th>
<th>Label</th>
<th>(M_n^a) (kDa)</th>
<th>(T_g^b) (ºC)</th>
<th>(T_{cp, heat}) (ºC)</th>
<th>(T_{cp, cool}) (ºC)</th>
<th>Hyst. (ºC)</th>
<th>95-5% T (ºC)</th>
<th>C (#)</th>
<th>O (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nPrPE</td>
<td>TR15</td>
<td>25.3</td>
<td>-20</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>iPrPE</td>
<td>TR16</td>
<td>17.1</td>
<td>-2</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>EtPE</td>
<td>TR17</td>
<td>58.0</td>
<td>6</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>MePE</td>
<td>TR18</td>
<td>45.3</td>
<td>-5</td>
<td>Insol</td>
<td>Insol</td>
<td>N/A</td>
<td>N/A</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>TR-EoMePE</td>
<td>TR19</td>
<td>75.4</td>
<td>-13</td>
<td>16(14)</td>
<td>8(7)</td>
<td>8(8)</td>
<td>3(2)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>TR-MoMePE</td>
<td>TR20</td>
<td>32.8</td>
<td>-9</td>
<td>61(63)</td>
<td>55(60)</td>
<td>6(3)</td>
<td>12(19)</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\)Determined by DMF SEC relative to PMMA standards. \(^b\)Determined by DSC. \(^c\)Determined by UV–vis for PE solutions in DI H₂O and (1X PBS).

As shown in Table 8.4, TR19 and TR20 underwent temperature-induced phase separation at 16 ºC and 61 ºC, respectively. This represented a spike in \(T_{cp}\) of 45 ºC by the removal of a single methylene and was similar to the large \(T_{cp}\) difference between TR11 and TR14. Similar to amide TR-PEs, the O/C ratio affected the \(T_{cp}\) of alkyl and alkoxy TR-PEs, with a main difference being oxygen atoms were required for the generation of a thermoresponsive material. Additionally, similar \(T_{cp}\) hysteresis was observed. However, the \(N\)-alkoxy TR-PEs did not seem to be greatly affected by the presence of salt, which suggests that the “salting-out” affect for TR-PEs is stronger for amide functional groups.
than alkoxy groups. This could be due to an increase in anion binding to the polymer, resulting in stronger polymer solvation which counteracts the increased hydrophobic effect and polarization of bound water by anions theorized as the cause of thermoresponsive polymer “salting-out.”\textsuperscript{21-24} Interestingly, TR20 was seen to show a broad phase transition and be significantly affected by molecular weight. The explanations for observations are not presently understood, but are likely similar to TR14.

8.5 Copolymerization Studies with Drug-containing Monomer

A key benefit of the modularity inherent to the TR-PE system is the virtually limitless number of copolymers that can be synthesized in order to generate a material with desired functionalities and physical properties. With the expanded library of TR-PE homopolymers, it is logical to assume that a copolymer with any desired LCST could be synthesized. This is especially useful if the desired comonomer is highly hydrophobic, but presents desirable functionality.

Previously, we reported that by changing temperature, TR-PEs could be used to physically encapsulate a hydrophobic small molecule model compound as a proof-of-concept example for possible applications within bio separations and drug delivery.\textsuperscript{212} However, the covalent attachment of hydrophobic drugs was not undertaken as the hydrophilicity of the TR-PEs was not high enough to counterbalance the addition. However, the expanded TR-PE library contains homopolymers such as TR14 and TR20 that show extremely high $T_{cp}$. When copolymerized with highly hydrophobic monomers, such as those bearing model drugs, it was hypothesized that a thermoresponsive polymer could still be attained.
To this end, TR 14 and TR20 monomers were selected for possible copolymerization studies as their homopolymers displayed the highest $T_{cp}$ in their respective class. Ibuprofen, a common nonsteroidal anti-inflammatory drug, was chosen as a hydrophobic model drug as its carboxylic acid functionality would allow for the attachment to DEA through a pendant hydrolysable ester bond, possibly releasing functional ibuprofen through hydrolytic degradation. As opposed to post-polymerization functionalization, using a drug-containing monomer allows for a high degree of control over drug incorporation with fewer synthetic steps. Thermoresponsive copolyesters were synthesized containing a 9 : 1 ratio of TR monomer to ibuDEA monomer which was confirmed via $^1$H NMR (Figure 8.5). TR-(90MoMe-$r$-10ibuDEA)PE ($M_n = 23.3$ kDa) and TR-(90MeEtA-$r$-10ibuDEA)PE ($M_n = 30.1$ kDa) displayed $T_{cp}$ at 8.8 °C and 17.0 °C, respectively. The difference in $T_{cp}$ of the similar molecular weight TR-PEs copolymers corresponded well to the relative hydrophilicities of the respective homopolymers. As the LCST of TR-PEs has been shown to be easily tuned via copolymerization, it can be expected that incorporating more or less ibuDEA monomer would shift the resulting $T_{cp}$ lower or higher. These data represent a significant step forward for the TR-PE system, as they show the feasibility of these thermoresponsive biodegradable materials for physical and covalent encapsulation of therapeutic, diagnostic, imaging, and targeting compounds. It can be imagined that such materials could display a number of release rates dependent on temperature, hydrolysis, coacervate polymer content, and polymer degradation. Currently, our group is investigating the release profile of physically and covalently attached ibuprofen from these copolymers.
Figure 8.5: $^1$H NMR spectra and UV–vis traces (inset) of TR-(90MoMe-r-10ibuDEA)PE (top) and TR-(90MeEtA-r-10ibuDEA)PE (bottom) (500 MHz, CDCl$_3$).
8.6 Conclusions

In this chapter, the synthesis and characterization of 20 PEs was described as to gain a better understanding of the pendant group role in physical, thermal, and thermoresponsive properties and possible implications thereof on copolymerization. The low $T_g$ of the PEs was seen to be most influenced by the presence of amide protons and all PEs showed good thermal stability. Of the 20 PEs, six new TR-PEs were added to the previously reported four to bring the total number of TR-PEs to 10 and the total $T_{cp}$ range to 0 – 100 ºC. It was determined that adding alkoxy substituents could increase the observed $T_{cp}$ of TR-PEs in a predictable manner using the O/C substituent ratio. Furthermore, TR-PE copolymers containing covalently attached ibuprofen were shown to be thermoresponsive. These results further expand the tunability of the TR-PE system and provide valuable insight for the design of future thermoresponsive biodegradable polymers, especially within the realm of smart polymeric drug delivery.
9.1 Introduction

Coacervation-type thermoresponsive polymers exhibit minimal conformational change when brought above their Lower Critical Solution Temperature (LCST) as compared to the drastic conformational changes witnessed with polymers that undergo efficient dehydration (and subsequent coil-globule transition) above the LCST. This is primarily due to the significant amount of water present in the coacervate phase and makes coacervate-type polymers ideal systems for the segregation of sensitive biomolecules (i.e. nucleic acids and proteins), whose biological function is dependent on maintaining their delicate structure.\textsuperscript{6, 8} Despite these benefits, far fewer examples of thermoresponsive coacervate-type polymers exist in literature as compared to coil-globule types. Even rarer are reports of thermoresponsive coacervate-type polymers capable of biodegradation.

It is much easier to encapsulate and release small, insensitive hydrophobic drugs as compared to therapeutically useful biomolecules such as proteins. Proteins are much more complex than hydrophobic small molecules as they contain hydrophobic, hydrophilic, and charged domains which must be balanced by corresponding domains on the desired deliv-
ery system. Additionally, the sensitive structure of the protein must not be affected by encapsulation. As such, this makes polymers capable of capturing/releasing active biomolecules the “holy grail” of encapsulation systems. A number of recent reports on thermo-responsive encapsulation of proteins exist. For example, Shea and coworkers were able to synthesize crosslinked anionic poly(NIPAM-co-acrylic acid-co-t-butylacrylamide) nanoparticles that could “catch-and-release” the cationic protein lysozyme due to nanoparticle domains capable of protein interaction. Additionally, Tirrell and coworkers have shown that complex coacervates formed by the sequential addition of oppositely charged polypeptides are capable of encapsulating charged proteins. However, to the best of our knowledge there exist no biodegradable materials that can encapsulate and release proteins by a simple thermo-responsive mechanism. Ideally, such a system would be based on a modular, coacervate-type polymer as to allow for non-damaging encapsulation and release of proteins with a variety of charge and size.

As discussed in previous chapters, biodegradable, thermo-responsive polyesters (TR-PEs) have been shown to be capable of thermally encapsulating therapeutic model compounds, making them attractive for applications within targeted drug delivery. A key advantage of the TR-PE system is the formation of polymer-rich coacervates above the LCST, which to the best of our knowledge is the only example of such a system prepared by step growth polymerization.

In this chapter, the incorporation of ionic groups into TR-PEs is explored. These studies further expand the usefulness of the TR-PE system for a number of biomedical applications, as many therapeutically relevant biomolecules, such as the aforementioned
proteins and DNA, are slightly charged at neutral pH.\textsuperscript{221-222} We hypothesize that the addition of ionic groups into the PE chain will allow them to associate with proteins below the LCST, resulting in physical encapsulation when brought above the LCST. As a result of TR-PE coacervation, the encapsulated proteins should be released without loss of structure with a decrease in temperature or as the PE coacervates degrade (Figure 9.1).

![Figure 9.1: Temperature driven encapsulation and release of protein by ionic TR-PEs.](image)

Our group has previously reported the synthesis of protected diol monomers bearing hydroxyl, amine, and carboxylic acid functionalities.\textsuperscript{9} Additionally, we have also shown that by changing the polyester diacid from succinic acid (SA) to glutamic acid (Glu), a cationic amine can be incorporated in the PE backbone without a reduction in the amount of functionalized diol. The addition of polar ionic groups is known to raise the LCST of thermoresponsive polymers. In order to balance the hydrophilic shift and tune the LCST to biologically relevant temperatures, a diverse set of relatively hydrophobic TR-PE monomers was explored for copolymerization. Additionally, the effect of biologically relevant concentrations of salt and pH on ionic TR-PE thermoresponsivity and protein encapsulation was probed.
Synthesis of Ionic Thermoresponsive Polyesters

The synthesis of $N,N$-bis(hydroxyethyl) $N$-succinamide (HESA) monomers has been discussed in detail in Chapters IV-VII and was undertaken according to a previous literature procedure. In the present Chapter, hydrophobic HESA monomers based on $c$-propylamine ($cPrA$), $i$-propylamine ($iPrA$), dibutylamine ($dBuA$), and piperidine (PPDA) were used to counterbalance the increased PE hydrophilicity by the highly polar ionic groups. $N,N$-bis(hydroxyethyl) $N$-(ionic)amide (HEA) monomers bearing Boc-protected ionic functionalities were synthesized according to previous reports by our group. Boc-protected Glu (BocGlu) was obtained from commercial sources. DPTS catalyzed carbodiimide-mediated polyesterification of HESA/HEA diols and succinic acid (SA)/BocGlu diacids led to a variety of TR-PEs bearing Boc-protected amine or carboxylic acid functionality. Alkyl urea formed during the polyesterification was removed by filtration and the TR-PEs were purified by precipitation into cold methanol to obtain pure polymer as an elastic solid. Purity was confirmed via $^1$H NMR and analyzed via SEC. The ratio of HESA/HEA monomer as well as the ratio of SA/BocGlu was easily tuned by monomer feed and quantified via $^1$H NMR. The Boc group was deprotected using 4M HCl in dioxane (Scheme 9.1). After removal of the HCl/dioxane under reduced pressure, deprotection was confirmed via $^1$H NMR in DMSO-d$_6$. 
Scheme 9.1: Synthetic route for preparation of ionic TR-PEs.

Figure 9.2: $^1$H NMR spectra of cPrA4 before (bottom, black) and after (top, red) deprote-ction showing complete removal of the Boc group. (500 MHz, DMSO-d$_6$).
9.3 Results and Discussion

A number of factors were explored for the successful design of ionic TR-PEs for protein encapsulation. Initial synthesis of ionic TR-PEs was based upon a hydrophobic $i$-propyl or $c$-propyl substituted amide HESA monomers. Based on previous reports, TR-iPrAPE homopolymer (iPrA1) was known to display a cloud point temperature ($T_{cp}$, an approximation of the LCST) of 7.8 °C in DI water. Likewise, the more hydrophobic analogue TR-cPrAPE homopolymer (cPrA1) was not observed to be water soluble unless surfactant or urea was added to the solution. It was hypothesized that these monomers, while initially believed to possess LCSTs too low for biomedical applications, would be useful in counterbalancing the polar ionic charge. In order to be biologically useful, ionic TR-PEs must not only be designed for use in neutral DI water; the effect of common biochemical additives such as salt and buffer on TR-PE solubility was probed. Additionally, solubility of ionic TR-PEs as a function of solution pH was explored as a result of the ionizable amine functional group. Furthermore, the amount of charge required for protein association had to be balanced with the increase in TR-PE solubility as well as possible increase in PE degradation rate. Finally, the affect of protein loading and complexation to ionic TR-PE was determined.

In order to explore the desired optimizations, a number of ionic TR-iPrAPE (iPrA1-4) and TR-cPrAPE (cPrA1-5) copolymers containing various amounts of Glu were synthesized (Table 9.1). Copolymerization with 10% BocGlu to generate TR-(iPrA-r-Glu)PE (iPrA2) increased the $T_{cp}$ by nearly 23 °C, although the the $T_{cp}$ was reduced by half when the solution is changed to PBS. This is likely a result of PBS salts reducing solubility of
the TR-PE similar to what was previously observed, but also due to screening of the cationic charge by the PBS salts. A similar effect was seen with 10% Glu in the more hydrophobic TR-(cPrA-r-Glu)PE (cPrA2), which exhibited a $T_{cp}$ at 12.5 °C in DI water but reduces to 5.3 °C in PBS. The thermoresponse of neither TR-PE seemed greatly affected by the addition of 10% (relative to TR-PE) bovine serum albumin (BSA), an anionic model protein, to the solution.

Table 9.1: Characterization of the solution properties of ionic Glu-TR-PEs

<table>
<thead>
<tr>
<th>Label</th>
<th>Monomer</th>
<th>$M_n^a$ (kDa)</th>
<th>Glu (%diacid)</th>
<th>PE : BSA (mg/mL)</th>
<th>Solution</th>
<th>$T_{cp}^b$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPrA1</td>
<td>iPrA</td>
<td>56.6</td>
<td>0</td>
<td>10 : 0</td>
<td>DI water</td>
<td>7.8</td>
</tr>
<tr>
<td>iPrA2</td>
<td>iPrA</td>
<td>45.2</td>
<td>10</td>
<td>10 : 0</td>
<td>DI water</td>
<td>30.0</td>
</tr>
<tr>
<td>iPrA2</td>
<td>iPrA</td>
<td>45.2</td>
<td>10</td>
<td>5 : 0</td>
<td>PBS</td>
<td>16.3</td>
</tr>
<tr>
<td>iPrA3</td>
<td>iPrA</td>
<td>45.2</td>
<td>10</td>
<td>5 : 0.5</td>
<td>PBS</td>
<td>17.6</td>
</tr>
<tr>
<td>iPrA3</td>
<td>iPrA</td>
<td>24.7</td>
<td>15</td>
<td>5 : 0</td>
<td>UP water</td>
<td>43.5</td>
</tr>
<tr>
<td>iPrA3</td>
<td>iPrA</td>
<td>24.7</td>
<td>15</td>
<td>5 : 0.5</td>
<td>UP water</td>
<td>44.7</td>
</tr>
<tr>
<td>iPrA4</td>
<td>iPrA</td>
<td>32.4</td>
<td>20</td>
<td>10 : 0</td>
<td>DI water</td>
<td>57.0</td>
</tr>
<tr>
<td>iPrA4</td>
<td>iPrA</td>
<td>32.4</td>
<td>20</td>
<td>5 : 0</td>
<td>PBS</td>
<td>31.5</td>
</tr>
<tr>
<td>iPrA4</td>
<td>iPrA</td>
<td>32.4</td>
<td>20</td>
<td>5 : 0.5</td>
<td>PBS</td>
<td>30.6</td>
</tr>
<tr>
<td>cPrA1</td>
<td>cPrA</td>
<td>25.5</td>
<td>0</td>
<td>10 : 0</td>
<td>DI water</td>
<td>Insol.</td>
</tr>
<tr>
<td>cPrA2</td>
<td>cPrA</td>
<td>46.1</td>
<td>10</td>
<td>10 : 0</td>
<td>DI water</td>
<td>12.5</td>
</tr>
<tr>
<td>cPrA2</td>
<td>cPrA</td>
<td>46.1</td>
<td>10</td>
<td>5 : 0</td>
<td>PBS</td>
<td>5.3</td>
</tr>
<tr>
<td>cPrA2</td>
<td>cPrA</td>
<td>46.1</td>
<td>10</td>
<td>5 : 0.5</td>
<td>PBS</td>
<td>6.6</td>
</tr>
<tr>
<td>cPrA3</td>
<td>cPrA</td>
<td>24.7</td>
<td>15</td>
<td>5 : 0</td>
<td>UP water</td>
<td>25.8</td>
</tr>
<tr>
<td>cPrA3</td>
<td>cPrA</td>
<td>24.7</td>
<td>15</td>
<td>5 : 0.5</td>
<td>UP water</td>
<td>26.2</td>
</tr>
<tr>
<td>cPrA4</td>
<td>cPrA</td>
<td>29.1</td>
<td>12.5</td>
<td>10 : 0</td>
<td>100 mM PB</td>
<td>10.0</td>
</tr>
<tr>
<td>cPrA5</td>
<td>cPrA</td>
<td>29.7</td>
<td>15</td>
<td>10 : 0</td>
<td>100 mM PB</td>
<td>14.1</td>
</tr>
</tbody>
</table>

$^a$Determined by DMF SEC relative to PMMA standards. $^b$Determined by UV–vis.
Figure 9.3: Temperature-dependent phase behavior of iPrA3 and cPrA3 (5 mg/mL) at pH 6-8 in the presence and absence of BSA (0.5 mg/mL) (500 nm, 1 ºC heating)

Similarly, BSA was observed to have little affect on solubility for TR-PEs containing 15% Glu when tested in ultrapure (UP) water (iPrA3 and cPrA3 at pH 7, Figure 9.3). However, pH was seen to have a fairly significant affect as the $T_{cp}$ of both polymers decreased ~15 ºC as pH increased from 6 to 8. This is a result of the highly polar charged amine deproto-nating at basic pH. The neutral amine is less polar than when charged, making the TR-PE more hydrophobic and causing a reduction in the LCST.

From the preceding observations, a number of generalizations were made in the attempt to optimize ionic TR-PE design. The charge on the ionic TR-PE was adjusted to allow for a resulting $T_{cp}$ between refrigeration and ambient temperature (5 – 20 ºC) to fa-cilitate easy protein association and encapsulation at body temperature (37 ºC). Highly salty solutions were avoided as the requisite amount of charge required to raise $T_{cp}$ would
likely increase TR-PE degradation. Solutions with varying pH were selected since they were known to modulate overall TR-PE charge and possibly the resulting protein interaction. To this end, cPrA was chosen as a target monomer as the overall hydrophobicity of the monomer was able to effectively balance a moderate amount of charge (12.5 – 15% Glu). Phosphate buffer (PB) was selected as the model solution, as it is a common biological media that contains much less solubility-affecting salt than 1X PBS. Cationic TR-(cPrA-r-Glu)PEs containing 12.5% Glu (cPrA4) and 15% Glu (cPrA5) were observed to display a $T_c$ of 10.0 °C and 14.5 °C, respectively. Fluorescently tagged BSA (FITC-BSA), was chosen as a model protein as it was hypothesized that the slight anionic charge would facilitate association with the cationic TR-cPrAPEs and allow for simple observation of thermally-induced coacervate encapsulation via fluorescence microscopy.

To dissolved TR-PE solutions containing 5 mg/mL TR-PE was added 0.5 mg/mL FITC-BSA, refrigerated at 4 °C for 30 min to facilitate polymer-protein association, and then incubated at 37 °C for 30 min to encourage thermoresponsive encapsulation. The resulting yellow solutions formed rich yellow coacervates after centrifugation (7500 x g, 10 min). Aliquots of FITC-BSA coacervates were spread on microscope slides and imaged. As shown in Figure 9.4, both cPrA4 and cPrA5 were able to encapsulate FITC-BSA as small highly fluorescent green coacervates are easily seen. Increasing the pH decreases the cationic charge of the TR-PE, resulting in a reduced encapsulation efficiency. This observation indicates the possibility of ionic TR-PEs releasing their payloads in response to pH. The reduction in encapsulation efficiency, particularly at pH 8, can be observed as an increase overall fluorescence as more FITC-BSA remains free of the coacervates.
Figure 9.4. Optical micrographs (20x magnification, FITC filter) of cPrA4 and cPrA5 co-acervates (5 mg/mL) in 100 mM PB containing FITC-BSA (0.5 mg/mL) at varying pH. Scale bar is 50 um.

After centrifugation of incubated TR-PE + FITC BSA solutions, the absorbance of the supernatant was recorded at 495 nm and compared to a FITC-BSA standard curve in order to better quantify encapsulation efficiency at 1% FITC-BSA loading. Using this method, it was determined that at pH 6 both cPrA4 and cPrA5 displayed an encapsulation efficiency greater than 74% (the maximum value for this assay). At pH 7 this value dropped to 23 ± 1% and 14 ± 1%, respectively. Protein encapsulation efficiency drops to less than 2% for
both TR-PEs at pH 8, indicating the encapsulation pH sensitivity of this system. As FITC photobleaches quickly, future experiments to quantify protein encapsulation efficiency would likely benefit from the use of total protein assays instead, such as the BCA or Bradford assay.

While the results from cPrA4 and cPrA5 show early promise for the use of TR-PEs as protein-encapsulation agents, they only allow for interaction with anionic proteins due to the use of BocGlu as the cationic component. Unfortunately, no simple protected-anionic diacid is cheaply and commercially available. Ideally, a polymer designed for protein encapsulation would be modular enough to accommodate proteins bearing overall cationic or anionic charge.

To this end, preliminary investigations into a second generation of ionic TR-PEs was explored. In this route, a HEA monomer bearing a protected carboxylic acid (mGlu) or amine (mLys) was copolymerized with an amide HESA (Scheme 9.1) and deprotected. In the first set of anionic polymers, the amount of charged diol was held constant at a high value 30% while the hydrophobicity of the HESA amide was varied. As seen in Table 9.2, this resulted in cPrA7 and PPDA1 exhibiting total solubility as a result of the significant amount of charge, while dBuA1 was too hydrophobic to solvate as a result of the dibutylamide pendant group.

In the second set of polymers, the previously investigated cPrA diol was copolymerized with varying amounts of anionic mLys diol (10-30%). As shown in Table 9.2, the $T_{cp}$ is shown to increase from 9.5 °C to 40.6 °C as the relative mLys is doubled from 10% to 20% total diol. Despite differences in molecular weight and UV–vis polymer concentration, the results for cPrA8 in PBS are somewhat comparable to the TR-cPrAPE containing
10% Glu (cPrA2) in PBS which showed a $T_{cp}$ at 5.3 °C. The slight increase in $T_{cp}$ is likely due to cPrA8, maintaining the same amount of charge as cPrA2, exchanging 10% of the cPrA diol for the more hydrophilic and cationic mLys diol, resulting in an increase in TR-PE hydrophilicity and increase in $T_{cp}$. Additionally, the primary mLys amine is more polar than the secondary Glu amine, which could explain the increased $T_{cp}$. These data suggest that while the two categories of ionic TR-PEs (backbone charge vs. pendant charge) are structurally different, similarities between the two may allow for easier optimization based on comparative results.

Table 9.2: Characterization of the solution properties of ionic HEA-TR-PEs

<table>
<thead>
<tr>
<th>Label</th>
<th>Monomer</th>
<th>$M_n^a$ (kDa)</th>
<th>mGlu (% diol)</th>
<th>$T_{cp}^b$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anionic TR-PEs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cPrA7</td>
<td>cPrA</td>
<td>56.6</td>
<td>30</td>
<td>Sol.</td>
</tr>
<tr>
<td>PPDA1</td>
<td>PPDA</td>
<td>45.2</td>
<td>30</td>
<td>Sol.</td>
</tr>
<tr>
<td>dBuA1</td>
<td>dBuA</td>
<td>45.2</td>
<td>30</td>
<td>Insol.</td>
</tr>
<tr>
<td><strong>Cationic TR-PEs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cPrA8</td>
<td>cPrA</td>
<td>24.7</td>
<td>10</td>
<td>9.5</td>
</tr>
<tr>
<td>cPrA9</td>
<td>cPrA</td>
<td>24.7</td>
<td>20</td>
<td>40.6</td>
</tr>
<tr>
<td>cPrA10</td>
<td>cPrA</td>
<td>32.4</td>
<td>30</td>
<td>Sol.</td>
</tr>
</tbody>
</table>

$^a$Determined by DMF SEC relative to PMMA standards. $^b$Determined by UV–vis in 1X PBS.

9.4 Conclusions

In this chapter, the synthesis and testing of ionic TR-PEs for protein encapsulation was described. By optimizing the pH, solution conditions, monomer, and amount of charge, two cationic TR-PEs based on TR-(cPrA-r-Glu)PE were shown to encapsulate fluorescently tagged BSA after simple mixing and an increase of temperature. Encapsulation efficiency was shown to be dependent on pH, with slightly acidic solutions resulting in greater polymer charge and subsequent encapsulation. To the best of our knowledge, these results represent the first examples thermoresponsive biodegradable polymers capable of
protein encapsulation simply by modulating temperature. It was determined that polymers bearing ionic pendant groups could display LCST-type behavior dependent on monomer and charge analogous to those bearing backbone charge. These results will serve as a launching point for the future studies of TR-PEs as drug delivery agents for sensitive biomolecule therapeutics.
In this publication, the synthesis and characterization of a new class of biodegradable, thermoresponsive polyesters (TR-PEs) based is described. The new polymers are based on N-functionalization of diethanolamine (DEA) via a simple transamidation reaction to generate a variety of diol monomers bearing amide, alkoxy, or alkyl functional groups. The resulting monomers were polymerized to high molecular weight via room-temperature carbodiimide-mediated polyesterification to create a library of homo- and copolymers.

Interestingly, the TR-PEs were shown to undergo inefficient dehydration above their Lower Critical Solution Temperature (LCTS) in aqueous solutions, resulting in the
formation of a stable coacervate phase as evidenced by DSC, UV–vis, DLS, and $^1$H NMR. The effect of solution additives such as urea, Hofmeister salts, and surfactant on TR-PE temperature-induced phase separation was investigated and found to be similar to that of other thermoresponsive polymers, particularly thermoresponsive polypeptides. As compared to many other PEs, the TR-PES exhibited accelerated degradation kinetics. Preliminary studies suggested that TR-PES showed minimal cytotoxicity, even at high concentrations, making them potential candidates for a number of biomedical applications.

The residue structure, primarily the oxygen/carbon ratio, was shown to play an important role in the physical, thermal, LCST, and coacervate composition of the copolymers. These properties could be easily and predictably tuned via copolymerization to generate TR-PES exhibiting cloud point temperatures ($T_{cp}$, an approximation of LCST) between 0 – 100 ºC.

![Figure 10.2: TR-PE library based on amide (Z) or alkyl & alkoxy (Y) residues.](image)

As proof of concept for drug delivery applications, TR-PES were shown to be capable of physical encapsulation of hydrophobic small molecule model compounds with an
increase in solution temperature as evidenced by fluorescence microscopy. Furthermore, copolymerization of highly hydrophilic monomers with hydrophobic monomers containing a model drug covalently attached via a hydrolysable linkage were shown to exhibit LCST-type behavior that could be modulated by comonomer hydrophilicity. Finally, as coacervate-type polymers have been shown to be ideal candidates for the encapsulation of sensitive biomolecule therapeutics, ionic TR-PEs were synthesized as possible candidates thermally-induced capture and release of proteins. After optimization of solution additives, pH, and TR-PE charge and residue structure, the preliminary investigations showed successful encapsulation of a fluorescently tagged model protein.

![Figure 10.3: Ionic TR-PEs for thermoresponsive protein encapsulation.](image)

The work presented in this dissertation represents a significant advancement to the previously described “peptide-like” PEs originally reported by our lab in 2013. First, few polymers exist that are capable of both aqueous thermoresponsivity and biodegradation
which typically suffer from a lack of functional presentations. In literature, only two examples to thermoresponsive, biodegradable coacervate-type polymers exist: as elastin-like peptides (ELPs) and poly(alkyl oxazolines) (PAOxs), which suffer from synthetic limitations (ELPs) and lack of diverse functionality (ELPs and PAOxs). The present highly modular, coacervate-forming, biodegradable TR-PE system is compatible with a diverse set of functional monomers and can be easily tuned via copolymerization as a result of the tolerant step-growth carbodiimide-mediated polyesterification mechanism. Furthermore, to the best of our knowledge no reports on biodegradable, coacervate-type polymers capable of thermoresponsive protein encapsulation exist, making TR-PEs attractive candidates for stimuli-responsive targeted drug delivery of sensitive biomolecule therapeutics.

In order to better understand the TR-PE system, future investigations would benefit from a better understanding of the dehydration mechanism and cooperativity, explored using temperature regulated $^1$H NMR, UV–vis, temperature modulated DSC, and molecular dynamics simulations as explored for other complex systems. Furthermore, the use of TR-PEs as drug delivery vehicles requires more detailed investigations into copolymer optimization, physical/ionic covalent encapsulation efficiencies, controlled and burst release kinetics, and confirmation of encapsulated and released protein structure via temperature controlled circular dichroism (CD) measurements. Additionally, more extensive toxicity tests on the degradation products of TR-PEs is required to further establish suitability for in vivo use.
BIBLIOGRAPHY


137. Emil, S. E.; Albert, P. R. Surgical sutures. 1967.


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

\[
\begin{array}{c}
\text{O} \\
\text{N} \quad \text{O} \\
\text{O} \\
\text{O}
\end{array}
\]

(ppm) 8 7 6 5 4 3 2 1 0 -1

2.11 2.01

4.13 6.86 6.51
CONTIN analysis of the DLS data of TR-PyrAPE ($M_n = 54.2$ kDa, $T_{cp} = 15.8$ °C) above and below the cloud point.

CONTIN analysis of the DLS data of TR-iPrAPE ($M_n = 56.5$ kDa, $T_{cp} = 7.8$ °C) above and below the cloud point.
1 wt% TR-(75PyrA-r-25iPrA)PE (T_{cp} ~12 °C), 5 drops 50 µM Nile Red in DMSO added to both tubes. Brought to RT w/ water bath for 1 min, photographed, centrifuged 3600 RPM for 5 min.
<table>
<thead>
<tr>
<th>Day</th>
<th>Sample 1 (kDa)</th>
<th>Sample 2 (kDa)</th>
<th>Sample 3 (kDa)</th>
<th>Avg $M_n$ (kDa)</th>
<th>Avg $M_n$ Remain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>72.7</td>
<td>72.7</td>
<td>72.7</td>
<td>72.7</td>
<td>100.0</td>
</tr>
<tr>
<td>1</td>
<td>58.9</td>
<td>56.9</td>
<td>59.9</td>
<td>58.6</td>
<td>80.6</td>
</tr>
<tr>
<td>4</td>
<td>38.5</td>
<td>36.8</td>
<td>36.8</td>
<td>37.4</td>
<td>51.4</td>
</tr>
<tr>
<td>7</td>
<td>27.2</td>
<td>27.3</td>
<td>25.6</td>
<td>26.7</td>
<td>36.7</td>
</tr>
</tbody>
</table>

Table S2. Degradation Analysis of TR-bMoEtAPE

<table>
<thead>
<tr>
<th>Day</th>
<th>Sample 1 (kDa)</th>
<th>Sample 2 (kDa)</th>
<th>Sample 3 (kDa)</th>
<th>Avg $M_n$ (kDa)</th>
<th>Avg $M_n$ Remain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>132</td>
<td>132</td>
<td>132</td>
<td>132</td>
<td>100.0</td>
</tr>
<tr>
<td>1</td>
<td>54.7</td>
<td>65.5</td>
<td>56.0</td>
<td>58.7</td>
<td>44.5</td>
</tr>
<tr>
<td>4</td>
<td>51.7</td>
<td>49.9</td>
<td>51.4</td>
<td>51.0</td>
<td>38.6</td>
</tr>
<tr>
<td>7</td>
<td>37.7</td>
<td>39.6</td>
<td>38.7</td>
<td>38.7</td>
<td>29.3</td>
</tr>
</tbody>
</table>