Precision Synthesis of Functional Materials via RAFT Polymerization and Click-Type Chemical Reactions

Joel Diez Flores
University of Southern Mississippi

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The University of Southern Mississippi

PRECISION SYNTHESIS OF FUNCTIONAL MATERIALS
VIA RAFT POLYMERIZATION AND CLICK-TYPE CHEMICAL REACTIONS

by

Joel Diez Flores

Abstract of a Dissertation
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

August 2011
ABSTRACT

PRECISION SYNTHESIS OF FUNCTIONAL MATERIALS VIA RAFT POLYMERIZATION AND CLICK-TYPE CHEMICAL REACTIONS

by Joel Diez Flores

August 2011

The need to tailor polymeric architectures with specific physico-chemical properties via the simplest, cleanest, and most efficient synthetic route possible has become the ultimate goal in polymer synthesis. Recent progress in macromolecular science, such as the discoveries of controlled/“living” free radical polymerization (CRP) methods, has brought about synthetic capabilities to prepare (co)polymers with advanced topologies, predetermined molecular weights, narrow molecular weight distributions, and precisely located functional groups. In addition, the establishment of click chemistry has redefined the selected few highly efficient chemical reactions that become highly useful in post-polymerization modification strategies. Hence, the ability to make well-defined topologies afforded by controlled polymerization techniques and the facile incorporation of functionalities along the chain via click-type reactions have yielded complex architectures, allowing the investigation of physical phenomena which otherwise could not be studied with systems prepared via conventional methods.

The overarching theme of the research work described in this dissertation is the fusion of the excellent attributes of reversible addition-fragmentation chain transfer (RAFT) polymerization method, which is one of the CRP techniques, and click-type chemical reactions in the precision of synthesis of advanced functional materials. Chapter IV is divided into three sections.
In Section I, the direct RAFT homopolymerization of 2-(acryloyloxy)ethyl isocyanate (AOI) and subsequent post-polymerization modifications are described. The polymerization conditions were optimized in terms of the choice of RAFT chain transfer agent (CTA), polymerization temperature and the reaction medium. Direct RAFT polymerization of AOI requires a neutral CTA, and relatively low reaction temperature to yield AOI homopolymers with low polydispersities. Efficient side-chain functionalization of PAOI homopolymers was achieved via reaction with model amine, thiol and alcohol compounds yielding urea, thiourethane and urethane derivatives, respectively. Reactions with amines and thiols (in the presence of base) were rapid, quantitative and efficient. However, the reaction with alcohols catalyzed by dibutyltin dilaurate (DBTDL) was relatively slow but proceeded to completion. Selective reaction pathways for the addition of difunctional ethanolamine and mercaptoethanol were also investigated.

A related strategy is described in Section II wherein a hydroxyl-containing diblock copolymer precursor was transformed into a library of functional copolymers via two sequential post-polymerization modification reactions. A diblock copolymer scaffold, poly[(N,N-dimethylacrylamide)-b-(N-(2-hydroxyethyl)acrylamide)] (PDMA-b-PHEA) was first prepared. The hydroxyl groups of the HEA block were then reacted with 2-(acryloyloxy)ethylisocyanate (AOI) and allylisocyanate (AI) resulting in acrylate- and allyl-functionalized copolymer precursors, respectively. The efficiencies of Michael-type and free radical thiol addition reactions were investigated using selected thiols having alkyl, aryl, hydroxyl, carboxylic acid, amine and amino acid functionalities. The steps of RAFT polymerization, isocyanate-hydroxyl coupling and thiol-ene addition are
accomplished under mild conditions, thus offering facile and modular routes to synthesize functional copolymers.

The synthesis and solution studies of pH- and salt-responsive triblock copolymer are described in Section III. This system is capable of forming self-locked micellar structures which may be controlled by changing solution pH as well as ionic strength. A triblock copolymer containing a permanently hydrophilic poly(\(N,N\)-dimethylacrylamide) (PDMA) outer block, a salt-sensitive zwitterionic poly(3\([2-(N\text{-methylacrylamido})\text{ethyl dimethylammonio}]\text{propanesulfonate}\)) (PMAEDAPS) middle block and a pH-responsive 3-acrylamido-3-methylbutanoic acid (PAMBA) core block was synthesized using aqueous RAFT polymerization. A facile formation of “self-locking” shell cross-linked micelles is achieved by changing solution pH and salt concentration. The reversible “self-locking” is attained from the interactions of zwitterionic groups in the middle block that constitutes the shell of the micelles. The structure slowly dissociates into unimers in 2-3 days at pH above the pKa of the PAMBA block.
ACKNOWLEDGMENTS

My sincerest gratitude goes to my research adviser, Dr. Charles L. McCormick, for allowing me to carry out my PhD research work in his laboratories. With his patience, mentoring, and support, I cherish the opportunity to work on projects that are interesting and currently relevant. I am also grateful of him for encouraging me to aspire for quality research and to not take shortcuts. I thank Dr. Gordon C. Cannon, Dr. Andrew B. Lowe, Dr. Sarah E. Morgan, Dr. Derek L. Patton and Dr. Marek W. Urban for their time, guidance and thoughtful discussions.

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Most importantly, I am thankful to my family and a few good friends for their support especially at times when giving up seemed the only option left.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>acrylic acid</td>
</tr>
<tr>
<td>ACPA</td>
<td>4,4′-azobis(4-cyanopentanoic acid)</td>
</tr>
<tr>
<td>ADVN</td>
<td>2,2′-azobis(2,4-dimethylvaleronitrile)</td>
</tr>
<tr>
<td>AEMA</td>
<td>2-aminoethyl methacrylate</td>
</tr>
<tr>
<td>AI</td>
<td>allylisocyanate</td>
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<tr>
<td>AIPD</td>
<td>4,4′-azobis[2-(imidazolin-2-yl)propane]dihydrochloride</td>
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<td>AlMA</td>
<td>allyl methacrylate</td>
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<td>3-acrylamido-3-methylbutanoic acid</td>
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<td>AMPS</td>
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<td>APE</td>
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<td>APMA</td>
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<tr>
<td>aRAFT</td>
<td>aqueous reversible addition-fragmentation chain transfer</td>
</tr>
<tr>
<td>ATRP</td>
<td>atom transfer radical polymerization</td>
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<tr>
<td>AzEMA</td>
<td>2-azidoethyl methacrylate</td>
</tr>
<tr>
<td>AzPAM</td>
<td>3-azidopropylacrylamide</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<tr>
<td>CAC</td>
<td>critical aggregation concentration</td>
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<tr>
<td>CEP</td>
<td>4-cyano-4-(ethylsulfanylthiocarbonylsulfanyl) pentanoic acid</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>CEV</td>
<td>2-(ethylsulfanylthiocarbonylsulfanyl)-2,4-dimethylvaleronitrile</td>
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<tr>
<td>CMP</td>
<td>2-(1-carboxy-1-methylethylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid</td>
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<tr>
<td>CRP</td>
<td>controlled/“living” free radical polymerization</td>
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<td>CSC</td>
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<td>CTA</td>
<td>chain transfer agent</td>
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<tr>
<td>EGMEMA</td>
<td>(ethylene glycol)methyl ether methacrylate</td>
</tr>
<tr>
<td>EMP</td>
<td>2-(ethylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid</td>
</tr>
<tr>
<td>EWG</td>
<td>electron-withdrawing group</td>
</tr>
<tr>
<td>FITC-Con A</td>
<td>lectin-fluorescein isothiocyanate conjugate</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>GMA</td>
<td>glycidyl methacrylate</td>
</tr>
<tr>
<td>GTP</td>
<td>group transfer polymerization</td>
</tr>
<tr>
<td>HEA</td>
<td>N-(2-hydroxyethyl)acrylamide</td>
</tr>
<tr>
<td>HEMA</td>
<td>2-hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>HPMA</td>
<td>N-(2-hydroxypropyl) methacrylamide</td>
</tr>
<tr>
<td>IPEC</td>
<td>inter-polyelectrolyte complex</td>
</tr>
<tr>
<td>LCST</td>
<td>lower critical solution temperature</td>
</tr>
<tr>
<td>MAEDAPS</td>
<td>3-[2-(N-methylacrylamido)ethyl(dimethylammonio)]propanesulfonate</td>
</tr>
<tr>
<td>MMA</td>
<td>methyl methacrylate</td>
</tr>
<tr>
<td>mPEO</td>
<td>(polyethylene oxide) methyl ether</td>
</tr>
<tr>
<td>NHS</td>
<td>N-hydroxysuccinimide</td>
</tr>
<tr>
<td>NIPAM</td>
<td>N-isopropyl acrylamide</td>
</tr>
<tr>
<td>NMP</td>
<td>nitrooxide-mediated polymerization</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PA</td>
<td>propargyl acrylate</td>
</tr>
<tr>
<td>PCL</td>
<td>poly(ε-caprolactone)</td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity</td>
</tr>
<tr>
<td>PE</td>
<td>polyelectrolyte</td>
</tr>
<tr>
<td>PMA</td>
<td>propargyl methacrylate</td>
</tr>
<tr>
<td>RAFT</td>
<td>reversible addition-fragmentation chain transfer</td>
</tr>
<tr>
<td>RI</td>
<td>refractive index</td>
</tr>
<tr>
<td>ROMP</td>
<td>ring-opening metathesis polymerization</td>
</tr>
<tr>
<td>SEC</td>
<td>size exclusion chromatography</td>
</tr>
<tr>
<td>SME</td>
<td>small molecule electrolyte</td>
</tr>
<tr>
<td>TBP</td>
<td>N,N-dimethyl-S-thiobenzoylthiopropionamide</td>
</tr>
<tr>
<td>TCEP</td>
<td>tris(2-carboxyethyl)phosphine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TMSPMA</td>
<td>trimethylsilylpropargyl methacrylate</td>
</tr>
<tr>
<td>UCST</td>
<td>upper critical solution temperature</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
</tbody>
</table>
CHAPTER I
INTRODUCTION

The recent discoveries of precise synthetic methods have boosted the field of macromolecular science, allowing the preparation of previously difficult, if not impossible, to make topologies via easier and simpler means. With the technology still in its relative infancy, polymer chemists, material scientists and engineers are facing substantial and exciting opportunities amidst other challenges such as the need for safer, more efficient and more environment-friendly products.\(^1\) With high precision synthesis, polymeric materials with programmable behavior can be used in highly demanding and advanced applications wherein utility of commodity polymers is considered inadequate.

Structure and function in polymers are closely interrelated. New materials with entirely different properties can result from the subtle manipulation of the polymer structures and of the functionality along the backbone or at the chain ends. Hence, one of the active areas in polymer science today delves with ways to control molecular attributes such as architecture, composition, chain length distribution, stereoregularity, block sequence, block length and precise location of reactive functional groups.\(^2\) Arguably, the two technological discoveries that have facilitated attainment of these goals are the development of a number of controlled polymerization methods and, recently, the establishment of click chemistry.\(^3-7\) These two methods in combination have proven to be highly useful in designing complex, multifunctional polymeric materials. These advanced macromolecular architectures make possible the investigation of physical phenomena and theories which otherwise could not be studied using systems prepared via conventional synthesis.\(^8-12\)
In this introductory chapter, reversible addition-fragmentation chain transfer (RAFT) polymerization, one of the most commonly used controlled / “living” free radical polymerization (CRP) techniques, is described. Furthermore, the general concepts and synthetic approaches utilizing click chemistry to further functionalize macromolecular structures are then explored. The discussion is followed by pertinent examples of syntheses as well as applications of well-defined stimuli-responsive (co)polymers.

Controlled/“Living” Free Radical Polymerization (CRP)

(Co)polymers with well-defined architectures are much more amenable to studies of structure-property relationships than those prepared by uncontrolled polymerizations. Thus, physicochemical properties can be targeted for applications that could not be attained previously. Since the pioneering work of Szwarc and coworkers in the 1950s, a number of synthetic methods including traditional ionic, ring opening (metathesis), and group transfer polymerizations, as well as the most recent CRP techniques, have been developed and utilized extensively in the preparation of complex architectures. Most CRP methods are based on the dynamic equilibrium between active and dormant species via either reversible activation/deactivation processes or degenerative chain transfer. The fast, dynamic equilibrium between the active and dormant species minimizes, if not eliminates, the occurrence of termination, chain transfer and other primary side reactions enabling the synthesis of (co)polymers having predetermined molecular weights and narrow molecular weight distributions. This is particularly critical in free radical-based polymerizations wherein growing chains can inherently terminate through disproportionation or bimolecular free radical-free radical coupling reactions. CRP achieves control through maintenance of sufficiently low and constant concentration of free radicals throughout the polymerization.
Scheme I-1. The dynamic equilibrium for NMP, ATRP and RAFT polymerization showing the reversible activation/deactivation or degenerative chain transfer between active and dormant chains (X is nitroxide for NMP, halide for ATRP and thiocarbonylthio for RAFT).

A number CRP methods provide simple and robust routes to the synthesis of well-defined, low polydispersity (co)polymers leading to the fabrication of novel functional materials. The most popular CRP systems include nitroxide-mediated polymerization (NMP), atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization. The dynamic equilibria for these three major CRP techniques are illustrated in Scheme I-1. In NMP, dormant polymeric alkoxyamines undergo homolytic cleavage to produce propagating free radicals and persistent nitroxide radicals. The propagating chains then add monomer and recombine with the persistent nitroxide radicals reverting back to the dormant chains. Recent developments have made NMP applicable to a wider, though still restricted, range
of monomers. In ATRP, reversible cleavage of covalently bound halides is accomplished via redox reactions catalyzed by transition metals. The oxidized metal complexes, like the nitroxides in NMP, serve as the persistent species readily accessible for the recombination with the propagating chains. ATRP is substantially more versatile than NMP; however, it requires unconventional initiating systems with poor compatibility with some polymerization media. More recent reports, however, have addressed this and other issues. The RAFT process involves degenerative chain transfer between propagating chains and chain transfer agents (CTAs) which are usually thiocarbonylthio-containing compounds. Chain propagation occurs by controlled addition of monomer to the propagating radicals. In all of these methods, ideally, the growing polymeric radicals should not irreversibly terminate in order to achieve a successful controlled / “living” polymerization. Various architectures (Figure I-1) afforded by these CRP techniques include homopolymers, alternating/ statistical/gradient/ block copolymers, grafts/combs, brushes, stars, functional telechelic copolymers as well as dendritic and branched topologies.
The RAFT Polymerization Process

Unlike NMP and ATRP, the RAFT technique operates on a degenerative chain transfer. Developed by CSIRO and first reported in 1998, this method is highly versatile as it allows polymerization for virtually all classes of vinyl monomers (i.e. (meth)acrylates, (meth)acrylamides, acrylonitriles, styrenics, butadienes, vinyl esters, vinyl amides, etc) under a variety of reaction conditions. A significant advantage is the excellent control afforded by RAFT for polymerization in aqueous media, a technique referred to as aqueous RAFT (aRAFT) polymerization. The control over molecular weight and polydispersity in RAFT polymerization is unaffected by the
presence of functional groups such as OH, NR₂, COOH, SO₃H, CONR₂. The essential features of an ideal RAFT polymerization are as follows:\(^{53}\)

(a) RAFT polymerization can be performed by simply adding a chosen quantity of an appropriate chain transfer agent (CTA) to a conventional free radical polymerization. In most cases, the same monomers, initiators, solvents and temperatures may be employed.

(b) RAFT polymerization possesses the characteristics usually associated with living polymerization. Essentially, chains begin to grow at the beginning of the polymerization and continue to grow until all the monomer is consumed.

(c) Molecular weights increase linearly with monomer conversion.

(d) Active chain ends are maintained allowing chain extension through addition of another batch of monomer.

(e) The molecular weights of the polymers in RAFT polymerization can be estimated using the initial monomer to CTA ratio and monomer conversion.

(f) Narrow molecular weight distributions are achievable.

(g) Blocks, stars and other complex molecular architectures are accessible depending on the CTA structure and order of monomer addition.
Scheme I-2. The proposed mechanism of reversible addition-fragmentation chain transfer (RAFT) process mediated by thiocarbonylthio-containing chain transfer agent (CTA).

**The Mechanism of RAFT Polymerization**

The generally accepted mechanism of RAFT polymerization is shown in Scheme I-2. The RAFT process uses the same sequence of steps as in classical free radical polymerization; however, a thiocarbonylthio-containing chain transfer agent (CTA) mediates the monomer addition to the chain. As a result, after the normal radical generation (initiation) and addition of the first monomer (initialization), a pre-equilibrium system is achieved in which addition of the formed radical to the CTA is followed by fragmentation of the S-R bond from the intermediate species 4 or 5. The reinitiation by \( \text{R}^\bullet \) (7) and subsequent addition by the resulting propagating species 8 to the dormant...
oligomeric or polymeric CTA (6 or 9) allow the development of the main equilibrium. Once the main equilibrium is established, degenerative chain transfer of the thiocarbonylthio species between the dormant (10) and growing chain $P_n\bullet$ or $P_m\bullet$ occurs through an intermediate free radical species 11. Propagation proceeds through the controlled addition of monomer to the growing polymer chains.

![Figure I-2](image-url) The generic chemical structures of chain transfer agents (CTAs) used in RAFT polymerization. The Z group for trithiocarbonate, xanthate and dithiocarbamate are $R'-S$, $R'-O$ and $R''(R')-N$, respectively.

As with other controlled pseudo-living polymerization techniques, the rate of initialization in RAFT polymerization is faster than the rate of propagation resulting in the activation of all CTA molecules before chains can start to grow. The equilibrium between the propagating polymeric free radicals and the dormant chains allows uniform chain growth since the intermediate can fragment in either direction. Thus, a successful RAFT polymerization will yield narrowly dispersed polymer. In principle, under optimal polymerization conditions, the total number of radicals is determined by the source of primary radicals and the number of chains is controlled by the concentration of CTA. The RAFT equilibrium effectively limits the number of irreversible termination events by minimizing the instantaneous concentration of primary free radicals. The number of dead chains after polymerization remains low and negligible (<5%).
Examples of compounds shown to mediate successfully the RAFT process include dithioesters, trithiocarbonates, xanthates and dithiocarbamates (Figure 1-2).\textsuperscript{41, 42, 47} The efficiencies of these chain transfer agents are dictated by the structures of the Z and R groups. The structure of the CTA must be chosen with great care in order to achieve the best control in RAFT polymerization of a particular monomer system. An excellent CTA has a high free radical chain transfer constant and a good reinitiating efficiency of the R group. The Z group plays important roles in activating the C=S bond as well as stabilizing the intermediate free radical. Longer lived intermediates can be achieved with highly stabilizing Z groups (e.g., phenyl). The rate of polymerization is inversely related to the lifetime of the intermediate free radical.\textsuperscript{55} On the other hand, the R group must readily undergo homolytic cleavage and subsequently add to a monomer to achieve an efficient degenerative chain transfer.\textsuperscript{56}

It should be noted that the RAFT polymerization mechanism can be extended to the formation of block copolymers by using a macroCTA. However, the order of monomer introduction when attempting block copolymerization must be carefully considered along with the choice of initiator, polymerization temperature and other experimental conditions. The propagating polymeric free radical (R group) of the first block must fragment and add to the second monomer efficiently to have successful chain extension.

Although controversy persists regarding the lifetime and reactivity of the intermediate species shown in Scheme I-2, proper selection of CTA structure, monomer and reaction conditions is vital to achieve full control over reaction kinetics, polymer molecular weight and molecular weight distribution. In addition, structoterminal or structopendent functionality may be precisely incorporated onto the polymer chain. A
number of reviews\textsuperscript{41-45, 47, 48, 51, 57} are available in literature detailing utility of the RAFT technique for the preparation of advanced architectures. General strategies in the synthesis of (co)polymers with desired and well-positioned functional groups will be described in the succeeding sections.

RAFT Polymerization Molecular Weight Control

According to the RAFT mechanism, polymer chains may be derived from the initiator fragments or the R group from the CTA. As such, the theoretical number-average molecular weight ($M_{n,th}$) of the polymer may be calculated as:

$$
M_{n,th} = \frac{[M]_0 M_{MW} \rho}{[CTA]_0 + 2f[I]_0(1-e^{-k_d t})} + CTA_{MW}
$$

where $[M]_0$ is the initial monomer concentration, $M_{MW}$ is the molecular weight of the monomer, $\rho$ is monomer conversion, $[CTA]_0$ is the initial concentration of CTA, $f$ is initiator efficiency, $[I]_0$ is the initial initiator concentration, $k_d$ is the decomposition rate constant of the initiator, $t$ is reaction time, and $CTA_{MW}$ is the molecular weight of the CTA. In typical RAFT polymerizations, the CTA to initiator ratio is kept high such that the concentration of free radicals remains low, thereby minimizing the number of dead polymer chains. Hence, equation (1) may be simplified into:

$$
M_{n,th} = \frac{[M]_0 M_{MW} \rho}{[CTA]_0} + CTA_{MW}
$$

By controlling conversion and the initial monomer to CTA ratio, well defined (co)polymers with predetermined molecular weights may be obtained.
RAFT Polymerization in Aqueous Media

Aqueous RAFT (aRAFT) polymerization, pioneered by the McCormick research group,\textsuperscript{43,51,52} has demonstrated excellent control and robustness in the synthesis of water-soluble polymers, a characteristic not readily achievable with any other controlled polymerization methods.\textsuperscript{50} As such, additional considerations must be noted to maintain control when conducting aRAFT polymerization. The thiocarbonylthio functional group of the CTA is susceptible to oxidation,\textsuperscript{58} hydrolysis,\textsuperscript{59} aminolysis\textsuperscript{60,61} as well as degradation by UV light.\textsuperscript{62} Oxygen free conditions are utilized in RAFT polymerization and therefore CTA oxidation is unlikely. Similarly, the reaction solution is typically exposed to a UV light source only when free radicals are generated photochemically. Hence, the effects of CTA degradation by UV light can be avoided by using azo-based thermal initiators. However, CTA degradation through aminolysis and hydrolysis must be avoided by utilizing buffers with RAFT polymerization in aqueous solutions.

Although thiocarbonylthio compounds are known to be thermodynamically unstable towards hydrolysis, there is a significant kinetic barrier to hydrolysis. Levesque and coworkers\textsuperscript{60} examined the hydrolytic stabilities of several thiocarbonylthio compounds and found that the rate of hydrolysis increases with temperature and solution pH. Similarly, Thomas et al.\textsuperscript{63} in the McCormick group studied the effect of solution pH on the hydrolysis of small molecule CTAs as well as macroCTAs. They reported that the rates of the hydrolysis of (4-cyanopentanoic acid)dithiobenzoate (CTP) and two macroCTAs of poly(sodium 2-acrylamido-2-methyl-1-propanesulfonate) (PAMPS) made with CTP increased with solution pH. Also, the small molecule CTA was shown to be more susceptible to hydrolysis, which was attributed to less steric hindrance as compared to the polymeric CTAs. Convertine and coworkers\textsuperscript{64} studied the effect of temperature on
trithiocarbonates, specifically 2-(1-carboxy-1-methyl-ethylsulfanyl thiocarbonylsulfanyl)-2-methylpropionic acid (CMP) and found that these species are more resistant to hydrolysis. They determined that at temperatures close 50 °C, hydrolysis of the trithiocarbonate is negligible for over 24 hours.

Aminolysis occurs when a primary or secondary amine reacts with the thiocarboxylthio moiety. This reaction is known to be first order with respect to the concentration of CTA and second order with respect to the amine concentration. Thomas et al. also conducted aminolysis experiments on CTP using ammonium hydroxide in buffered media. After 4 hours, over 95 % of CTP is degraded emphasizing the importance of solution pH in aRAFT polymerizations. Furthermore, aRAFT polymerizations of (meth)acrylamido monomers should be carried out under slight-to-moderate acidic conditions, as these monomers may undergo hydrolysis themselves. Given the high monomer concentrations relative to that of the CTA, a few percent of monomer hydrolysis can easily result in NH₃ that ultimately degrades the CTA. Moderately acidic conditions, which minimize both hydrolysis and aminolysis, are necessary in order to retain the thiocarbonylthio moiety.

Initially, monomers containing primary or secondary amines were considered incompatible with RAFT polymerization. However, by lowering the solution pH to keep the amino groups protonated, CTA aminolysis may be avoided. For example, Li et al. reported the first successful aRAFT polymerization of the primary amine containing monomer N-(3-aminopropyl)methacrylamide (APMA) by maintaining the solution pH between 4 and 5. Similarly, a report by Alidedeoglu et al. also detailed the controlled polymerization of a primary amine containing monomer, 2-aminoethylmethacrylate (AEMA) in an acetate buffer at pH 5. Armes and coworkers also reported the RAFT
polymerization of AEMA in DMSO using cumyldithiobenzoate as the CTA. Successful chain extension of mPEO macroCTA with APMA directly in water at pH 4-5 was reported by the McCormick group.\textsuperscript{68}

Click Chemistry Concept

A decade ago, Kolb, Finn and Sharpless\textsuperscript{69} first coined the term “click chemistry” in open literature. These authors defined a set of stringent criteria that a chemical reaction must meet to be considered a click chemistry. Such reactions must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by nonchromatographic methods (e.g. crystallization, distillation, etc) and be stereospecific. The required characteristics also include simple reaction conditions and use of readily available starting materials. These select few reactions are “spring-loaded” due to high reagent reactivity and low activation barriers and thus proceed rapidly to completion, ideally yielding a single product. The following are the classes of chemical reactions suggested to have met the criteria of click chemistry.\textsuperscript{69}

(a.) cycloaddition of unsaturated species, especially 1,3-dipolar cycloaddition reactions as well as Diels-Alder family of transformations;

(b.) nucleophilic substitution chemistry, particularly ring-opening reactions of strained heterocyclic electrophiles such as epoxides, aziridines, aziridinium ions, and episulfonium ions;

(c.) carbonyl chemistry of the non-aldol type, such as formation of ureas, thioureas, aromatic heterocycles, oxime ethers, hydrazones, and amides; and

(d.) addition to carbon-carbon multiple bonds, especially oxidative cases such as epoxidation, and sulfonyl halide addition, dihydroxylation, aziridination, sulfonyl halide addition as well as Michael additions of nucleophilic reactants.
While the origins of chemistry involved in “click” type reactions go back several decades, the term coined ten years ago largely refers to polymer conjugation reactions of biologically active molecules. However, applications of these reactions have had a profound effect on the polymer field as indicated by the number of peer reviewed scientific publications, since 2001 (Figure I-3). In addition to the requirements described above, Barner-Kowollik and others\textsuperscript{70} suggested that the application of click chemistry in polymer synthesis should include the use of equimolar amounts of reactants specially in polymer-polymer conjugation since removal of the unreacted species becomes a challenge. But if large scale purification is not an issue, an excess of one of the starting materials may be employed to enhance yields over a reasonable timescale.\textsuperscript{70}
Figure I-4. Stepwise mechanism proposed for the Cu(I)-catalyzed 1,3-dipolar cycloaddition of azides and alkynes (L-ligand, B-base).\textsuperscript{71,72}

**Azide-Alkyne Coupling**

Perhaps, the most well-known click chemical reaction is the 1,3-dipolar cycloaddition of azides and alkynes which has evolved recently into a common coupling procedure across various chemical disciplines.\textsuperscript{73-79} In the absence of transition metal catalyst, this coupling reaction that forms a triazole ring is rather slow and, in most cases, not regioselective.\textsuperscript{72,80,81} However, the use of catalytic amount of copper (I) that binds to terminal alkynes leads to fast, highly efficient and regioselective azide-alkyne cycloaddition at room temperature both in organic as well as protic reaction media.\textsuperscript{82} The proposed mechanism for the Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition is shown in Figure I-4.

The potential toxicity of metal catalysts used in azide-alkyne cycloadditions or any other chemical reactions, in general, can be a major drawback when the resulting products are to be used in bio-related applications.\textsuperscript{83-85} Hence, there has been significant interest in developing alternative click reactions that do not require any metal catalyst.\textsuperscript{86} A variation of azide-alkyne cycloaddition that does not require the addition of copper
catalyst but proceeds relatively fast and high conversion under mild conditions is the use of activated and/or strained alkynes.\textsuperscript{87-94} The strained-promoted azide-alkyne cycloaddition may be enhanced by incorporating electron-withdrawing groups on the ring.\textsuperscript{95} For example, the presence of gem-difluoro group adjacent to the strained alkyne led to reactions with azides that were 30-60 times faster than those with non-fluorinated analogues.\textsuperscript{96}

\textit{Diels-Alder Cycloaddition}

As a concerted pericyclic reaction, Diels-Alder (4+2) cycloaddition involves simultaneous breaking and formation of carbon-carbon bonds (see Figure I-5).\textsuperscript{97-100} The reaction itself has low energy requirement and may be carried out at ambient conditions depending on the structures of the dienophile and the diene reactants. The dienophile, which can be activated by Lewis acids, may contain an electron-withdrawing group conjugated to the carbon-carbon double bond. The diene reactant, on the other hand, may be open-chain or cyclic and may be substituted as well. The substituent groups affect the molecular orientation of the reactants in the transition state, and hence, the stereochemistry of the product.\textsuperscript{101} Being highly efficient and specific, Diels-Alder reactions exhibit the characteristic qualities of click chemistry and have been used extensively in synthesis of various architectures such as dendrimers, stars, networks and functional telechelic copolymers.\textsuperscript{86, 102-109}
Diels-Alder Cycloaddition

Thiol-Ene Addition (free radical)

Thiol Michael Addition

Isocyanate Coupling Reactions

Figure I-5. Examples of chemical reactions that meet the criteria of click chemistry.

Thiol-Ene Additions

The coupling of thiols and alkenes, whether via free radical (termed as thiol-ene reaction) or anionic mechanism (termed as thiol Michael addition), carry the attributes of click chemistry (Figure I-5).\textsuperscript{110-115} The scope of these hydrothiolation reactions is extremely impressive with virtually any thiol and any alkene yielding quantitative products under very mild reaction conditions. This click reaction is especially attractive due to its simplicity, high precursor reactivity and availability of a broad variety of starting materials. In addition, thiol-containing proteins, glycoproteins and other bio-
relevant species can undergo facile thiol-ene coupling, providing a facile route for preparing polymeric bioconjugates.

The photochemically or thermally-induced thiol-ene addition proceeds by a free radical mechanism to give an anti-Markovnikov thioether product. The rate of addition is influenced by the chemical structure of the alkene with electron-rich and/or strained alkenes reacting more rapidly than electron-poor alkenes (e.g., norbornene > vinyl ether > alkene ≈ vinyl ester > allyl ether > acrylate > N-substituted maleimide > methacrylate > conjugated dienes). Driven by the high nucleophilicity of the thiolate anion, thiol Michael addition may be carried out with activated alkenes (i.e., alkenes attached to electron-withdrawing groups) in the presence of organobases (e.g., tertiary amines) and nucleophiles such as primary and secondary amines as well as certain tertiary phosphines. Furthermore, the overall rate of thiol Michael addition is affected by the solvent polarity and pH (for reactions in solution), strength of base catalyst, pKa and steric bulkiness of the thiol, and the nature of the electron-withdrawing group (EWG) on the alkene.

Isocyanate-Based Click Chemistries

The chemistry of urea (NCO + amine) or thiourethane (NCO + thiol) formation was identified by Sharpless and coworkers as one of the non-aldol type carbonyl reactions that meet the criteria of click chemistry. As with the other click chemistries, the high efficacy, utility and absence of by-products that are associated with these two reactions have been reported in literature (Figure I-5). However, the isocyanate-based click reactions are the least explored to date in both academic as well as industrial laboratories. The urea-forming reaction of amines with isocyanates does not require the addition of a catalyst, as amines are sufficiently nucleophilic to react with the isocyanate group. Thus, the relative rates of reaction depend on the nucleophilicity of the
attacking amine. In the absence of a catalyst, thiol-isocyanate reactions occur very slowly, at a rate slower than that of the alcohol. However, with the addition of a base catalyst, the reaction proceeds rapidly and efficiently. The base first deprotonates the thiol and the formed thiolate anion then adds to the isocyanate, followed by proton abstraction to form the thiourethane.

Synthetic Approaches to Advanced Functional Polymers

So far, the discussion covers the fundamentals of CRP, specifically RAFT polymerization, and representative examples of click chemical reactions. In this section, general approaches and examples of reports available in literature regarding the synthesis of pendent and end-functional (co)polymers utilizing RAFT polymerization and click reactions are described.

While the molecular weight, polydispersity and structure of polymers may be tailored by selection of appropriate reagents and polymerization methods, the precise positioning of reactive groups along the polymer chain needed in later transformations is a challenging task. Usually, this can be accomplished in two ways: (a) direct incorporation of monomers, chain transfer agents and/or initiator fragments that already contain the target functionality and (b) post-polymerization modification of reactive polymeric precursor to transform terminal or pendent groups into the desired moieties.

Direct Polymerization of Functional Monomers

The direct polymerization of functional monomers gives very high functional densities and guarantees the presence of the functional group at each repeating unit. The traditional living anionic and cationic polymerizations offer very limited possibilities for direct polymerization of functional monomers. However, the developments of CRP techniques as well as those of catalytic polymerizations provide alternative routes with
better functional group tolerance. In spite of these, there remain a number of
functionalities that cannot be introduced by direct polymerization using any currently
available polymerization methods. Functional groups such as free amines, thiols and
carbon-carbon multiple bonds may either completely prevent controlled polymerization
or may participate in side reactions that can lead to loss of control over the
polymerization process. It should be pointed out that primary amine-containing
monomers have been successfully polymerized by RAFT under acidic conditions where
the amines are protonated.\textsuperscript{65-68} Therefore, direct polymerization may require time-
consuming protecting group chemistry if the functional group interferes with the
polymerization method. It should be noted that the additional deprotection step may not
necessarily proceed to completion and, hence, may affect the structural integrity of the
polymer backbone. Lastly, another concern for the direct polymerization approach is the
commercial availability and cost of reagents.\textsuperscript{6}

\textit{Post-Polymerization Modification of Polymers}

Post-polymerization modification is an attractive route for the synthesis of
functional polymers that can overcome the limited functional group tolerance of many
controlled/living polymerization techniques. This approach requires the polymerization
of monomers having moieties that are inert towards the polymerization conditions but
which can be quantitatively converted in a subsequent reaction step into a broad range of
desired functional groups.\textsuperscript{4} However, the chemical reaction utilized in the post-
polymerization modification must be highly efficient; otherwise, it cannot guarantee that
each reactive group is successfully converted into the desired functionality. Resolving
functionalized macromolecules from unreacted or partially reacted ones may be difficult,
if not impossible to achieve.\textsuperscript{6} Furthermore, these transformation reactions must be highly
specific, facile and robust so as to prevent side reactions with other moieties that might be present in the system. Thus, click chemical reactions have found great utility in post-polymerization modification strategies.  

**Pendent Functional Polymers**

Precursor (co)polymers prepared from polymerization of monomers having the clickable functional group can be modified via grafting-to approach to obtain pendent functional (co)polymers. However, some clickable groups cannot be tolerated in CRPs. For example, terminal alkynes are known to be chemically and thermally unstable in the presence of free radicals. Indeed, reports have shown that polymerizations of vinyl monomers containing free alkyne functionality have resulted in side reactions such as free radical addition to the triple bond, chain transfer, complexation of the alkyne with the catalyst and insertion reactions that lead to crosslinking.  

Ostaci and colleagues prepared random copolymers of propargyl methacrylate (PMA), glycidyl methacrylate (GMA) and methyl methacrylate (MMA) via RAFT polymerization. The resulting alkyne- and glycidyl-containing pseudobrushes were uncontrolled having broad polydispersities (PDI=1.6-2.0). Similarly, copolymerization of propargyl acrylate (PA) and acrylic acid (AA) also resulted in high polydispersions. However, Zhang and others reported successful RAFT block copolymerization of (ethyleneglycol)methyl ether methacrylate (EGMEMA) and PMA with polydispersities that were less than 1.3. These block copolymers were then functionalized with fluorescent hydrophobic groups yielding amphiphilic systems that were capable of forming micelles and vesicles depending on the block lengths.

As mentioned in the preceding paragraphs, the use of protecting group chemistry might seem to negate the efficiency of the overall process; but the best way to prepare
polymeric precursors containing the alkyne functionality for post-polymerization modification utilizes the trialkylsilyl protecting group. Using cyanoisopropyl dithiobenzoate as the CTA, the Stenzel research group polymerized protected PMA via RAFT to afford a polymer backbone with each repeating unit bearing the alkyne functionality. After deprotection, narrow polydispersity comb copolymers were obtained by reacting the pendent alkyne with an azide end-functionalized poly(vinyl acetate). Withey and coworkers reported the preparation of poly[(trimethylsilylpropargyl methacrylate)-b-(poly(ethylene glycol) methyl ether methacrylate)] via RAFT polymerization. After removal of the protective group, the amphiphilic copolymer self-assembled into nanoparticles (< 20 nm). The pendent alkyne could be used as a reactive group for crosslinking and also as ligand for complexation with cobalt ion. The encapsulated cobalt complexes, which are antitumor agents, reduced undesirable toxicity.

As an alternative route, a functional monomer with a pendant azide moiety, 2-azidoethyl methacrylate (AzEMA), was polymerized via RAFT process with excellent control over the molecular weight distribution (PDI =1.05–1.15). The subsequent copper-catalyzed cycloadditions of phenyl acetylene were achieved at room temperature with high conversions. The resulting functional polymer exhibited nearly identical \(^1\)H NMR and FT-IR spectra compared to a polymer of the same molecular structure but prepared by a pre-functionalization approach, confirming the retention of the azide group. A doubly hydrophilic diblock copolymer, poly(N,N-dimethyl acrylamide)-b-poly(N-isopropyl acrylamide-co-3-azidopropylacrylamide) (PDMA-b-P(NIPAM-co-AzPAM)) containing azide moieties was synthesized via consecutive RAFT polymerizations. The diblock copolymer molecularly dissolved in aqueous solution at room temperature, and self-assembled into core-shell nanoparticles above the lower critical solution
temperature (LCST) of the P(NIPAM-co-AzPAM) block. Core cross-linking was facilely achieved upon addition of difunctional propargyl ether. The obtained core cross-linked micelles possessed thermo-responsive cores; and the swelling/shrinking of the micelles could be finely tuned with temperature, rendering them as excellent vehicles for drug delivery. A highly efficient room temperature synthetic route to prepare intramolecularly cross-linked nanoparticles from poly[(methyl methacrylate)-co-(3-azidopropyl methacrylate)-co-(3-trimethylsilyl-propyn-1-yl methacrylate)] terpolymers was reported by Loinaz and coworkers. The resulting nanoparticles were further functionalized through reaction of the excess azide groups with propargyl glycine. It should be noted that azides are known to be sensitive to heat and UV light. Hence, thermally-initiated polymerization as well as photopolymerization may not be suitable in the preparation of azide-containing polymers. Azides can undergo cycloaddition reactions to carbon double bonds at higher temperatures. Bai and coworkers reported successful living free radical polymerization of azide-containing polymers at room temperature using γ irradiation as well as redox initiation.

Schlaad and coworkers utilized thiol-ene additions to functionalize butadiene homopolymers and block copolymers by reacting the pendent alkenes with small molecule thiols containing carboxylic acid, primary and tertiary amines, hydroxyl, glucose, esters, cholesterol, benzyl and fluorinated groups. These post-polymerization modification reactions provide significant changes in the chemical and physical characteristics of the substrate (co)polymers. The resulting amphiphilic block copolymers showed a wide range of solution properties and were responsive to changes in temperature, pH or electrolyte concentration. The carboxylate-modified polybutadiene-$b$-poly(ethylene oxide) copolymers rendered the system glass-like, reducing the
propensity to desorb from the surface; the functionalization with oligopeptides resulted in worm-like micelles or vesicles in aqueous solutions. The free radical thiol-ene addition to polybutadienes is accompanied by intra-molecular cyclization reactions that reduce the efficiency of the thiol conjugation to less than 85%. In contrast, intra-molecular cyclization was not observed in thiol-ene additions of polyoxazolines-bearing pendent alkenes and derivatized (co)polymers which have more flexible and longer spacers between the backbone and the pendent alkene groups.

Temperature-responsive block neoglycopolymers were prepared via sequential post-polymerization modifications by Chen and coworkers. Firstly, a block copolymer of PEGMEMA (n=2) and 2-hydroxyethylmethacrylate (HEMA) was prepared by RAFT polymerization; subsequent esterification of the pendent hydroxyl groups with 4-pentenoic anhydride followed by thiol-ene addition using glucothiose yielded functional copolymers that can be thermally-triggered to form micelles. From direct RAFT polymerization, Bulmus and coworkers also functionalized alkene-containing diblock copolymers of allyl methacrylate (AlMA) and N-(2-hydroxypropyl)methacrylamide (HPMA) with cysteamine via photoinitiated thiol-ene additions. The resulting cationic copolymers were investigated as potential carriers in gene delivery applications.

Pendent group transformations provide a variable number of sites for conjugation as determined by the number of repeating units within the block. Yet, modification of pendent groups via thiol-ene click chemistry cannot be readily carried out due to the difficulty of preparing the polymer precursors with pendent thiols or alkenes. Some reports show that by judicious choice of alkene reactivities, well-defined polymers with pendent alkenes can be prepared from selective polymerization of asymmetric
bifunctional vinyl monomers using CRP methods.\textsuperscript{157-160} However, this can only be achieved at relatively low monomer conversions (\(<50\%\)) or by statistical copolymerization with other monomers. Chain extension to make pendent alkene-functionalized block copolymers remains a challenge without losing control of polymerization. Most attempts to polymerize such monomers result in branched or cross-linked structures.\textsuperscript{161-166} Employing ring-opening and ionic polymerizations, well-defined polymer precursors with pendent alkene groups can be directly prepared.\textsuperscript{143, 144, 146, 150, 167-174} However, these polymerization methods require metal catalyst, are conducted under stringent reaction condition, and are less tolerant to functional groups and impurities as compared to CRP techniques. Hence, there is a need for better routes for the synthesis of polymeric precursors for thiol-ene modification.

\textit{Telechelic Functional Polymers}

End-functional groups on polymers serve as strategic starting points for the synthesis of a significant number of more complex structures. For example, two functionalized polymers, each possessing one chemically unique end-group capable of reacting only with the chain end on the other polymer, allow for covalent coupling to afford a diblock copolymer. This polymer-polymer coupling is particularly useful in the preparation of diblock copolymers wherein the constituent monomer for each block is not polymerizable by the same method.\textsuperscript{109} Additionally, end-functional homopolymers can be used in the synthesis of multiblock copolymers, graft copolymers, star-shaped architectures, and cross-linked networks.\textsuperscript{3, 6, 7, 57} The success of this strategy is dependent upon the high fidelity of end group incorporation and efficiency of the coupling reaction.\textsuperscript{3} The synthesis of end-functional polymers can be accomplished via initiators or chain transfer agents that already contain the desired reactive groups. In the case of RAFT
polymerization, reactive moieties such as azides, protected thiols, activated esters, strained heterocyclics and carbon-carbon multiple bonds (Figure I-6) may be attached to the Z or R groups of the CTA to afford $\alpha$ or $\omega$ end-functionalized polymers, respectively. The polymerization method must remain unaffected by these reactive handles on the initiators and CTAs.

Figure I-6. Examples of RAFT chain transfer agents with clickable reactive groups which are used in the synthesis of end-functional (co)polymers. 

$^57$
Telechelic or dually functionalized polymers can generally be classified into two structural types. The first has the same functional group at both chain ends (i.e., homobifunctional), while for the second class, the functional groups at the α- and ω-chain ends are different (i.e., hetero-bifunctional). Three basic strategies have been developed for the preparation of these systems. The first involves the use of a bifunctional initiator, which after polymerization and termination or chain end modification, affords a homobifunctional telechelic polymer. The second strategy uses functional initiators that provide the desired reactive groups at the α-chain ends, and coupling through the ω-chain ends of two homopolymers results in telechelic polymers with molecular weights that are twice that of the starting end-functional homopolymers. The final method employs functional initiators or CTAs in the polymerizations and the desired reactive moieties are added via quenching with terminating agents or modifying the functionality at one of the chain ends.

Polymers with carboxylic acids as chain end groups are easily obtained via RAFT polymerization. These carboxyl end-functional polymers can then be easily conjugated to other polymers, oligopeptides, carbohydrates and various molecules via traditional coupling reactions. Traditional coupling reactions in bioconjugation, however, have several drawbacks including multiple steps, difficulty in purification and reaction efficiency. To improve yields, activated ester RAFT agents were developed by several groups (Figure I-6). For example, Tew and colleagues modified CTP with N-hydroxysuccinimide (NHS) utilizing carbodiimide coupling. The resulting CTA provided good control over the polymerization of 4-vinyl benzoic acid. Similarly, the Theato research group prepared a RAFT agent and an azo free radical initiator, both containing a pentafluorophenyl activated ester (PFP), to mediate polymerization of
MMA, PEGMEMA, HPMA, and lauryl methacrylate, giving homopolymers and diblock copolymers with good control over molecular weights and molecular weight distributions. Polymers derived from the PFP-RAFT approach possessed $\alpha$-functionality that could be reacted with amines in high efficiency. Wiss and others demonstrated the utility of the PFP-RAFT for the bioconjugation of polymer with a collagen peptide.\(^{182}\)

Two new epoxy and oxetane functional RAFT agents (Figure I-6) able to control the polymerization of several acrylic monomers were reported by Vora and coworkers.\(^{183}\) The epoxy end group was modified by reaction with amines and carboxylic acids. The oxetane group was copolymerized with 3-ethyl-3-hydroxymethyl oxetane in the presence of $\text{BF}_3\cdot(C_2\text{H}_5)_2\text{O}$ as a catalyst, yielding trithiocarbonylthio macromonomers. These epoxy and oxetane functionalities hold great promise for polymer bioconjugations as they can be used in subsequent click reactions.

RAFT CTAs bearing norbornenyl, mono- or bisallyl, and cinnamyl groups were also described.\(^{184, 185}\) Allyl groups are of particular interest since they can be exploited for modification via thiol-ene addition leading to more complex architectures. Maleimide terminated polymers were also obtained using a furan-protected maleimide RAFT agent.\(^{186}\) The protecting group was cleaved (retro Diels-Alder reaction) by heating the polymer at 110 $^\circ\text{C}$ to yield maleimide-terminated poly(oligoethylene glycol acrylate) (POEG-A) with a functionality equal to 60-80%. Thiol-functionalized lysozyme was then conjugated to the polymer via thiol Michael addition to the maleimide end group.

The Sumerlin research group\(^{187, 188}\) reported the synthesis of an azido-terminated PNIPAM via RAFT polymerization and the thermo-responsive homopolymer was then conjugated to an alkyne-functionalized bovine serum albumin (BSA) protein. Similarly, a RAFT agent bearing an azide and a dithiopyridine group at its R and Z fragments,
respectively, was reported by CAMD group and successfully used in the RAFT polymerization of styrene, NIPAM and OEG-A. The heterotelechelic functionality of the polymers was proven by the successful conjugation of PNIPAM to alkyne-modified biotin and thiol-bearing glutathione and BSA via selective chemistries. Heterobiofunctionalized PNIPAM, (i.e., α-biotin, ω-BSA), was further modified via affinity interactions of biotin with the protein avidin.

Rather than performing the functionalization after polymerization, Zhao and Perrier opted for the attachment of the molecule of interest to the CTA before carrying out polymerization. This strategy allows good control over the architecture of the polymer–peptide conjugates, providing polymeric chains with a high degree of end group functionality. Similarly, utilizing thiol-maleimide coupling, De and coworkers functionalized a BSA protein with a RAFT agent which was subsequently utilized in the polymerization of NIPAM at room temperature. The resulting conjugate exhibited behavior which was reliant upon the responsive nature of the immobilized polymer. A related approach was also described by Boyer and coworkers.

With the potential toxicity concerns of the thiocarbonylthio groups in RAFT polymers, various ways of its removal after polymerization are reported in literature. Several groups have converted the thiocarbonylthio functionality of the CTA to free thiols and other reactive groups which can be utilized in end-group conjugation. For example, the McCormick research group described the reduction of the CTA moiety in RAFT polymers through aminolysis and the subsequent conjugations of fluorescent tags via disulfide exchange and amide formation as well as via thiol-maleimide coupling. Simultaneous aminolysis of various RAFT polymers and thiol-ene additions with small molecule alkenes were reported by Davis and
The one-pot approach prevented the formation of disulfide inter-chain cross-linking. The addition of thiol-terminated homopolymers into diacrylate or dimethacrylate monomers yielded macromonomers for graft copolymerization. Finally, the group also showed the facile attachment of bio-relevant species (i.e., biotin, mannose, oligodeoxyribonucleoside). Recently, the Lowe and Hoyle research groups also investigated the end-group transformations of RAFT polymers via thiol-Michael addition, thiol-isocyanate coupling and free radical thiol-ene click reactions. Polymer-protein conjugation using small molecule coupling agent was demonstrated by Li and colleagues. Thiol-terminated PNIPAM was first reacted with excess of 1,8-maleimido diethyleneglycol and the resulting maleimide-terminated polymer was subsequently conjugated to BSA or ovalbumin through reaction of the cysteine residues.

In order to utilize the highly efficient click reactions in the synthesis of end-functional polymers, the polymer has to be prefunctionalized with the appropriate moieties necessary for the transformations. This entails a special preparation for the CTA or initiator and possibly alteration of polymerization conditions to maintain control over polymer molecular weight and polydispersity as well as minimizing side reactions. Recently, Barner-Kowollik and Stenzel reported an elegant strategy of directly employing RAFT polymers in polymer-polymer conjugation without the need for prefunctionalization. A RAFT polymerized polystyrene (PS) was conjugated to a diene-terminated poly(ε-caprolactone) (PCL) in a hetero Diels-Alder cycloaddition. In this atom-efficient approach, the dithioester serves as the CTA in the RAFT polymerization and as the reactive heterodienophile in the polymer-polymer coupling. The group also extended this approach to the synthesis of 3- and 4-arm star polymers using multi-functional diene cores. The cycloadduct linkages were reversible and the
arms of the stars were completely cleaved when the polymers were heated at 160 °C for 24 hours.

The utility of controlled polymerizations and click chemistry is not restricted to the synthesis of pendent and end-functional (co)polymers. Extensive efforts have been directed towards utility of these methodologies in preparing supramolecular assemblies, networks and other complex topologies as well as in modifying solid surfaces for a number of advanced applications.  

![Figure I-7](image)

**Figure I-7.** Reversible self-assembly of block copolymers in solution into micelles triggered by the application of an external stimulus such as a change in temperature, pH or ionic strength.

**Stimuli-Responsive Polymers**

Smart polymers exhibit a directed, and ideally, detectable response that is induced by externally applied stimuli. These stimuli could be changes in temperature, solution pH and concentration. Additionally, application of mechanical force, interaction with chemical species, and irradiation with light, electric, magnetic or sonic energy can also trigger responses of smart polymers. Such responses of polymers can be a change in the chain dimension, secondary structure, degree of inter-molecular association and, in certain cases, breaking or formation of chemical bonds. These physical and chemical
events result in the alteration of secondary interactions (i.e., H-bonding, hydrophobic association, electrostatic interactions), occurrence of chemical reactions between groups present on the polymer chain (e.g., acid-base neutralization, redox reactions, etc), and changes in the concentration of certain species. Smart polymeric materials are utilized in applications such as controlled release, biosensing/diagnostics, separations, electronics, formulations, enhanced oil recovery and water remediation, among others.\textsuperscript{209-211}

Smart polymeric structures, such as block copolymers with one of the blocks responsive to a certain stimulus, possess interesting assembly characteristics in bulk and at interfaces as well as in polymer solutions. The polarities, dimensions and inter-chain associations of these smart copolymers, and hence the formation of their self-assembled nanostructures, may be altered by introduction of external stimuli (Figure I-7). With interesting and unique properties and, hence a wide array of potential applications, stimuli-triggered supramolecular assemblies such as micelles, vesicles, bioconjugates, films, networks and patterned surfaces have been prepared using smart polymers prepared via controlled polymerization methods.\textsuperscript{210} The most common smart polymeric materials include those that are responsive to changes in temperature, ionic strength and solution pH.

\textit{Temperature-Responsive Polymers}

Temperature-responsive (co)polymers exhibit a volume phase transition at a critical temperature, which causes a sudden change in the solvation state. Polymers that become insoluble upon heating exhibit a lower critical solution temperature (LCST). Conversely, systems that become soluble upon heating have an upper critical solution temperature (UCST). Thermodynamically, the LCST and UCST behavior of polymers can be explained as a balance between entropic effects of the dissolution process
involving the ordering/disordering of water molecules at the vicinity of the polymer chain and the enthalpic effects originating from hydrogen bonding, hydrophobic association and electrostatic interactions. Generally, these coil-to-globule transitions are manifested macroscopically as changes in polymer solubility in a given solvent system. A typical example of temperature-responsive polymer is PNIPAM.\textsuperscript{212,213} Synthesis through CRP methods as well as applications of PNIPAM homopolymers and copolymers are extensively reported in literature.\textsuperscript{214} Other temperature sensitive systems include analogues of NIPAM such as N-(n-propyl)acrylamide, \(N, N\)-diethylacrylamide, and \(N\)-ethylmethacrylamide; derivatives of amino acid L-proline; \(N\)-acryloylpyrrolidine; \(N\)-vinyl pyrrolidone; and \(N\)-acryloylpireredine.\textsuperscript{48}

\textit{Salt-Responsive Polymers}

Polyelectrolytes (PEs) are (co)polymers with repeating units that are permanently charged. The repulsion of the similarly charged groups along the chain provides PEs with extended chain conformations in solutions. However, when small molecule electrolytes (SMEs) are added to PE solutions, the ions from SMEs screen the repulsive forces between the charged groups along the backbone, causing the polymer chain to have a more collapsed conformation. The extent of this behavior, termed as polyelectrolyte effect, is contingent upon the identity of the charged groups on the polymer as well as the identity and concentration of the added SME.\textsuperscript{215,216} The majority of the PE systems are based on vinylic monomers (e.g., (meth)acrylamides, (meth)acrylates, and styrenics) containing sulfonated or quaternized amino groups.\textsuperscript{48}
**pH-Responsive Polymers**

Polymers containing ionizable groups belong to a class of pH-responsive polyelectrolytes. These may be weak polyacids or weak polybases. As the solution pH is changed, the degree of ionization of the polymer causes a change in the solubility of the chain, and therefore its hydration state, often leading to polymer aggregation. The pH-responsive anionic polyelectrolytes such as polyacrylates and polymethacrylates are protonated at pH values below their pKa, rendering the polymer hydrophobic and insoluble. However, the polymers become hydrophilic and water-soluble when the carboxylic acid groups are ionized at higher pH values. On the other hand, polybases are protonated at pH values below their pKa and are neutral at higher pH values. Reversible protonation and deprotonation of the ionizable groups via adjustment of solution pH are extensively exploited in the preparation of pH-controlled nanostructures.

Over the last couple of decades, the number of reports in open literature dealing with the direct preparation of well-defined copolymers from CRP and other controlled polymerization methods as well as the characterization and application of their stimuli-triggered assemblies are increasing exponentially. A number of reviews have summarized the recent developments as well as challenges associated with this research area.

**Click Chemistry Modifications**

In this section, examples of studies from literature involving controlled polymerization methods in combination with click chemical reactions to prepare stimuli-responsive materials are described. Specific reports on structoterminal and structopendent group transformations to modify polymers functionalities for controlling solution properties as well as providing moieties for efficient cross-linking of nanostructures are
summarized. Lastly, efficient strategies for encapsulation of toxic drugs, surface functionalization of nanoparticles and bioconjugation involving stimuli-responsive polymers are also briefly discussed.

Utilizing highly efficient and specific click chemistries, post-polymerization modifications can result in a library of functional (co)polymers from a single precursor, saving valuable time and resources compared to separately synthesizing each of the desired functional polymer. One of the immediate outcomes of transforming the pendent or terminal groups of a polymer is the dramatic change in its solubility characteristics. In a series of studies, Schlaad and coworkers demonstrated that conjugation of thiols having polar groups to a hydrophobic polybutadiene backbone via thiol-ene addition yielded amphiphilic polymers capable of forming nanoassemblies in aqueous media. Functionalization of polybutadiene-\(b\)-PEO with fluorinated thiols led to the formation of multicompartment micelles. The group also reported the conjugation of monosaccharides and oligopeptides to polybutadiene block copolymers and the resulting amphiphiles were shown to yield various morphologies (e.g., spherical micelles, wormlike micelles and vesicles) which could be tuned by controlling the hydrophilic/hydrophobic balance. Derivatization of polybutadienes with cationic (primary amine) and anionic (carboxylic acid) groups led to the formation of ionically stabilized inter-polyelectrolyte complexes (IPECs). With the difficulty of directly polymerizing monomers having bulky chromophoric group, Zhang and coworkers described the synthesis of alkyne-functionalized diblock copolymer. This precursor was then reacted with pyrene-containing azide. The formation of micellar and vesicular structures from the resulting amphiphilic copolymer was subsequently probed via fluorescence spectroscopy. Small molecule end-group conjugation via click reactions of
thermo-responsive PNIPAM and PDEA homopolymers, synthesized via RAFT, altered the polymer LCSTs. The stimuli-induced assembly of block copolymers in solutions yields supramolecular structures which may be utilized to capture, protect, and/or deliver active agents. However, these nanoassemblies can dissociate spontaneously back into unimers when polymer concentrations fall below the critical aggregation concentration (CAC), resulting in burst or premature release of the load, and thus, limiting practical applications. Therefore, a number of cross-linking methodologies including photochemical reactions, carbodiimide coupling, quaternization of tertiary amines with alkyl halides, oxa-Michael addition, activated ester substitution reactions as well as reversible linkages such as oximes, disulfides and inter-polyelectrolyte complexes (IPEC) have been developed to stabilize and control the assembly and disassembly of these structures. Li and colleagues prepared thermally-responsive ABC triblock copolymer, PEO-b-(DMA-stat-N-acryloyloxy succinimide)-b-PNIPAM. Upon formation of micelles above the LCST of PNIPAM, the activated ester groups in the middle block were reacted with ethylenediamine yielding covalently cross-linked micelles that swelled as the temperature was brought below the LCST of PNIPAM. When cystamine was used instead of ethylenediamine, the cross-links became reversible and degradable as the additional disulfide bridges were sensitive to redox active agents such as tris(2-carboxyethyl)phosphine (TCEP) and dithiothreitol (DTT). Conversely, a cleavable, temperature-responsive polymeric cross-linker was utilized by Xu and coworkers to stabilize micelles from PEO-b-PAPMA-b-poly((N,N-diisopropylamino)ethyl methacrylate) (PEO-b-PAPMA-b-DPAEMA) triblock copolymer. The PNIPAM cross-linker was prepared via RAFT polymerization using CMP, a bifunctional and
symmetrical CTA. The terminal carboxylic acid groups were then converted into N-hydroxy succinimidyl ester and utilized in the cross-linking process through reaction with the primary amine in the APMA middle block. Being susceptible to aminolysis, the trithiocarbonate moiety located at the middle of the cross-linker, can be degraded to break the cross-links. The preparation of single chain intra-molecularly cross-linked nanoparticles (< 20 nm) from a polymer system containing both pendent azides and alkynes was reported by Loinaz and coworkers. The residual azido groups were reacted with propargyl glycine to functionalize the nanoparticles with zwitterionic groups needed for stabilization in aqueous solution.

The disadvantage of treatment using small molecule drugs is the systemic distributions leading to unwanted side effects and low efficacy. Drug administration is improved using carriers, which allow not only a temporal control of the drug release, but also provide a targeted delivery. With this as a goal, Stenzel and coworkers designed a delivery vehicle wherein the inorganic antitumor agents were complexed into the core of the nanoparticles. The stealth protection through shell cross-linking via azide-alkyne click reaction resulted in reduced drug toxicity. Thermo-responsive synthetic glycopolymers were reported by Chen and colleagues via thiol-ene click addition. The facile conjugation of glucose into the polymer scaffolds afforded self-assembled particles having specific affinity to lectins. The binding of saccharides to lectins may be exploited in the targeted delivery of drugs and other diagnostic agents. Similarly, with cancerous cells known to overexpress folate-receptors at their surfaces, De and colleagues described the conjugation of folic acid to the terminal of thermo-responsive block polymers via azide-alkyne click cycloaddition. Utilizing primary amine-
containing triblock copolymers, York and colleagues demonstrated the facile conjugation of folic acid through the activated ester route.\textsuperscript{221, 222}

The preparation of smart polymer-protein conjugates is attractive and useful because the responsive nature of the polymer can be conferred to the substrate to which it is attached. Therefore, such design provides a handle wherein the activity or stability of the conjugated biomolecule may be controlled. In a number of reports,\textsuperscript{187, 192, 207} Sumerlin’s research group established facile methodologies for conjugating stimuli-responsive polymers to model proteins. In one study, a maleimide-containing CTA was conjugated to the protein and subsequently used in RAFT polymerization of NIPAM. The group also demonstrated the use of thiol Michael addition and an azide-alkyne click to make bioconjugates via “grafting to” approach.

![Figure I-8](image)

**Figure I-8.** General structures of (a) carboxybetaines, (b) sulfobetaines and (c) phosphobetaines.

**Zwitterionic Polymers**

In the preceding section, applications of click chemistry to functionalize or modify the properties of stimuli-responsive materials are described. These post-polymerization modification strategies are particularly critical whenever the target functionality and other moieties affect the control of polymerization and when the
appropriate functional monomers are not available. Nevertheless, there are also
significant number of reports in literature on the direct synthesis of functional stimuli-
responsive (co)polymers. This synthetic route is not discussed further in
this dissertation. However, synthesis of zwitterionic polymers, which is a unique class of
stimuli-responsive materials, is briefly described in this last section.

General Properties of Polybetaines

Polymer scientists have always been interested in the synthesis of functionalized,
well-defined zwitterionic polymers which are considered excellent mimics for naturally
occurring zwitterionic biomolecules such as carbohydrates, proteins and polynucleotides.

Synthetic zwitterionic polymers, which are comprised of either polybetaines or
polyampholytes, contain both anionic and cationic charges and exhibit unique solution
properties based upon the distribution of charges along the chain. Each repeating unit of
polybetaines contains both a positive and a negative charge, while polyampholytes have
combinations of positively and negatively charged monomers. Depending on the relative
number of the cationic and anionic monomers, the overall charge of a polyampholyte
may be neutral, positive, or negative. On the other hand, the overall charge of a
polybetaine is neutral but it can be adjusted to positive, or negative using pH responsive
moieties such as carboxylic acid or amino groups.

Three types of polybetaines commonly reported in literature include: (a)
carboxybetaines, (b) sulfobetaines and (c) phosphobetaines (Figure I-8). The negative
charges on sulfo- and phosphobetaines are permanent in common pH ranges because they
are weaker bases compared to a carboxylate anion. The cationic moiety may be made pH
sensitive by employing primary, secondary or tertiary amines. Through addition of an
acid or a base, pH sensitive polybetaines can be converted into polyelectrolytes.
The intra- and inter-molecular attractive forces between the zwitterionic groups in polybetaines form ionic network that limit the solubility of polybetaines in aqueous solutions. Furthermore, these interactions provide polyzwitterions collapsed conformations. The network structures can be disrupted by the addition of SMEs.\textsuperscript{223} The addition of salts results in an “anti-polyelectrolyte effect” (APE) that gives chains more extended, random conformation. The inherent difficulty of dissolving polybetaines in organic solvents or at low ionic strength aqueous media has been the major obstacle limiting studies in this field. Both attractive and repulsive forces exist in polyampholytes. However, some polyampholytes may show a combined behavior of polyelectrolytes and polybetaines depending on the chain composition.

Unlike polyelectrolytes, polybetaines in dilute aqueous solutions have minimal effect on the bulk water network structure because of the closely positioned charges that favor chain-chain interactions.\textsuperscript{224-227} Controlling the solubility of polybetaines has been studied since Morawetz and Ladenheim\textsuperscript{228} reported the first synthesis of a carboxybetaine in 1957. A number of factors determine the solubility of polybetaines. Being a charged solute, a polybetaine is more soluble in protic solvents that are capable of hydrogen bonding. For most systems, a critical salt concentration (CSC) is needed to maintain solubilization by disrupting the strong intra- and inter-polymer interactions. The solubilizing capacity for cations and anions follows the Hofmeister lyotropic series.\textsuperscript{223, 224, 229-232} The disruption of the chain-chain interactions of betaine units is surprisingly different for cations and anions.\textsuperscript{229} The slopes of the plots of reduced viscosities as a function of polysulfobetaine concentration added with LiCl, NaCl and KCl did not vary significantly. However, those with KCl, KBr and KI changed dramatically. The small effect of cations may be due to the charge to radius ratio.\textsuperscript{230} Smaller cations have greater
surface charge density and bigger hydration shell compared to anions with more diffuse electron clouds. The former may therefore have some difficulty in approaching the negatively charged moieties on the polymer chain. However, one must be judicious in choosing salt to solubilize polybetaines because certain ions (for example SCN⁻ on a polycarboxybetaine) may cause phase separation by binding tightly to the zwitterionic unit and causing eventual dehydration of the chain.²³³

The distance between the charges greatly affects the dipole moment of the monomer unit. At larger distances, stronger coulombic attractions exist between oppositely charged groups, resulting in lower solubility.²³⁴,²³⁵ Having more than four methylene units between the charges also renders the polybetaine hydrophobic.²²⁷,²³⁶ For a series of small molecule aminoalkanoic acid salts, their apparent pKa’s in water increase from 2.5 to 4.5 when the methylene spacer between the amino and the carboxylate groups is varied from 1 to 6.²³⁷ The amino group neutralizes the anion to a certain extent making it more stable, and hence, a weaker base. Although it can be more easily deprotonated, the resulting zwitterion has a decreased dipole moment, and therefore, has weaker electrostatic interactions.²³⁸ Similar characteristics are observed for polybetaines.²³⁹,²⁴⁰

While commonly reported polybetaines utilize amines as the cationic group, the anionic groups exhibit different interactions and solubilities. In general, carboxy- and phosphobetaines have better solubilities than sulfobetaines.²²³ Protonation of carboxylate anion in carboxybetaines occurs due to the weak acidity of carboxylic acids.²²⁴,²²⁷ Carboxybetaines often contain a slightly cationic net charge resulting from protonation of carboxylate anions. If this occurs, the chain exhibits polyelectrolyte characteristics and becomes more soluble.
Polybetaines modified with hydrophobic groups may self-assemble in solution and microphase-separate in bulk. The balance of hydrophobic and hydrophilic interactions dictates the self-assembly and phase-separation processes. The type of hydrophilic head group and geometry of the ionic groups determine the solution properties. Polyphosphobetaines with hydrophobic tails have drawn much attention as a biomimic for phospholipid bilayers (i.e., liposomes) and as drug delivery vehicles. Unlike small surfactants, fluoroalkyl end-capped polysulfobetaines exhibit unique solution properties. Extraordinary phase stability in bulk and interesting aggregation behavior in solution were observed in betaine end-functionalized polymers.

Diblock polyphosphobetaines based on phosphorylcholine monomers and NIPAM reversibly self-assemble in response to changes in solution temperature. Di- and triblock copolymers of sulfobetaine and DMA exhibit electrolytic-responsive assembly in water. Likewise, the self-assembly of block copolymers of sulfo- and phosphobetaine monomers and hydrophobic comonomers is affected by the addition of SMEs. Selective post-polymerization modification of tertiary amine-containing polymers affords polysulfobetaines which are pH responsive. Biocompatible drug delivery vehicles have been made from the copolymers of phosphobetaine and pH-responsive alkyl methacrylates. The micelles formed are of appropriate size and colloidal stability with pH-modulated drug uptake and release.

**Synthetic Approaches to Polybetaines**

Detailed reviews of the synthesis of polybetaines are available in literature. Polycarboxybetaines can be synthesized via direct polymerization of betaine monomers or through modification of polymeric precursors. Generally, pendent tertiary amine groups are reacted with strained lactones, 1,2-unsaturated carboxylic acids,
haloalkylcarboxylates or haloalkyl esters followed by hydrolysis of esters to yield carboxybetaines with quaternized amines as the cationic group. Similarly, the tertiary amines of monomer or polymer precursor may be reacted with sultones (1,3-propanesultone or 1,4-butanesultone) or haloalkysulfonates to give polysulfobetaines. The reactions with strained lactones and sultones are preferred because they generate halide free polycaboxylbetaines and polysulfobetaines, respectively. Cycopolymerization of $N,N$-diallylammonium derivatives results in pyrrolidinium type polybetaines. Analogues of this type have been reported by various research groups. \cite{17, 236, 255-261}

(Meth)acrylamido or (meth)acrylate derivatives are also common in the synthesis of polybetaines. \cite{91, 233, 235, 239, 248-250, 262} Polybetaines based on vinylpyridine\cite{228} and vinylimidazole\cite{229} have also been synthesized. A number of synthetic routes for the synthesis of polyphosphobetaines are described by Lowe and McCormick.\cite{223} The anionic center is usually at the tail end of the pendant group in carboxy- and sulfobetaines. However, in phosphorylcholine-based polybetaines, the anionic center is located at the middle of the side chain while the cationic group is at the chain end (Figure I-8). Although more expensive than the other two types of polybetaines, polyphosphobetaines have attracted much interest due to their phospholipid-like structures and have been employed in designing biocompatible polymers for biomedical applications.\cite{247, 249, 253, 263}

Most research to date on the synthesis of polymeric betaines has employed conventional free radical polymerization. The presence of oppositely charged groups requires the addition of SMEs to prevent precipitation. While free radical polymerization suffices for some applications, formation of monodisperse polymeric micelles or vesicles requires that unimers have narrowly dispersed amphiphilic block structures.\cite{143, 218} Solubility problems, strong electrostatic interactions, and lack of tolerant polymerization
methods have limited the synthesis of monodisperse polybetaines until the advent of CRP methods.

Polybetaines with narrow distributions can be prepared by post-polymerization modification of appropriate polymers synthesized \textit{via} group transfer polymerization (GTP) or NMP. Post-polymerization modification can lead to incomplete derivatization\textsuperscript{43}, thus direct synthesis of the betaine monomers is desired. Direct polymerization using ATRP\textsuperscript{247, 249, 253, 264-268} or RAFT polymerization\textsuperscript{223, 269-274} has been reported for carboxy-, phospho- and sulfobetaines. ATRP synthesis mostly employs protic organic solvents. The RAFT technique, on the other hand, allows polymerization betaine monomers in homogeneous aqueous media. This is particularly useful for the synthesis of less soluble polybetaines which require pH adjustment and higher electrolyte concentrations.
CHAPTER II

OBJECTIVES OF RESEARCH

Currently, the need for highly sophisticated polymeric architectures is increasing; and the ability to tailor these defined structures in the simplest, cleanest and most efficient means possible has become the ultimate goal in polymer synthesis. Recent progress in macromolecular science such as the discoveries of controlled/”living” free radical polymerization (CRP) methods has brought about synthetic capabilities to prepare (co)polymers with advanced topologies, predetermined molecular weights, narrow molecular weight distributions, and precisely located functional groups. In addition, the establishment of click chemistry in 2001 has redefined the selected few highly efficient chemical reactions, becoming one of the most versatile elements in a polymer chemist’s toolbox. The ability to prepare well-defined functional (co)polymers by direct polymerization or through post-polymerization modification of reactive precursor has yielded complex architectures, allowing the investigation physical phenomena which otherwise could not be studied with systems prepared via conventional synthesis.

The overarching theme of the research work described in this dissertation is the fusion of the excellent attributes of controlled polymerization techniques and click-type chemical reactions in the synthesis advanced functional materials. Specifically, reversible-addition fragmentation chain transfer (RAFT) polymerization is used to prepare (co)polymeric precursors via direct polymerization of reactive monomers without resorting to protecting group chemistry. Facile coupling of isocyanates with alcohols, amines and thiols as well as thiol-ene click addition reactions are utilized in post-polymerization modification to attach various functional groups along the (co)polymer backbone. Characterization of the resulting copolymers, their stimuli-induced self-
assembly and stabilization of the nanostructures through cross-linking are also described. With excellent efficiency and robustness, it is envisioned that these synthetic strategies will contribute to the methods available for the synthesis complex architectures.

This dissertation is divided into three sections. Firstly, the direct RAFT homopolymerization of 2-(acryloyloxy)ethylisocyanate (AOI) and subsequent post-polymerization modifications are described. The reactions between the pendant isocyanate groups and small molecule compounds having amine, alcohol or thiol groups are a facile means to functionalizing PAOI homopolymer. A related strategy is described in the second section wherein a hydroxyl-containing diblock copolymer precursor was transformed into a library of functional copolymers via two sequential post-polymerization modifications. In this case, the pendant hydroxyl groups were reacted with bifunctional alkene-isocyanate linkers yielding alkene-functionalized copolymer precursors. Selected thiols having alkyl, aryl, hydroxyl, carboxylic acid and amine functionalities were then conjugated to the structo-pendent alkenes via either Michael-type or free radically-mediated thiol-ene addition reactions. Lastly, the synthesis and solution studies of dually responsive triblock copolymer are described in the third section. This system is capable of forming self-locked micellar structures which may be controlled by changing solution pH as well as ionic strength.

The specific objectives of this research are the following:

1. Directly polymerize 2-(acryloyloxy)ethylisocyanate (AOI) monomer without protecting groups via the RAFT radical process;

2. Determine the reaction parameters in AOI RAFT polymerization that yield the best control over molecular weight, polydispersity and maintenance of pendant isocyanate functionality;
3. Demonstrate facile side-chain modification of PAOI homopolymer using click-type reactions between the pendant isocyanate group and model amine, alcohol and thiol compounds;

4. Synthesize precursor copolymer scaffold, poly[(N,N-dimethylacrylamide)-b-(N-(2-hydroxyethyl)acrylamide)] (PDMA-b-PHEA), and perform post-polymerization modification by reacting the pendent hydroxyl groups with 2-(acyroyloxy)ethylisocyanate (AOI) and allylisocyanate to form alkene-functionalized copolymers;

5. Investigate the efficiencies of Michael-type and free radical thiol-ene addition reactions of structopendent alkene-containing copolymer scaffolds using selected thiols;

6. Synthesize well-defined doubly-responsive triblock copolymer, PDMA-b-PMAEDAPS-b-PAMBA using RAFT polymerization directly in aqueous media;

7. Investigate the self-assembly of PDMA-b-PMAEDAPS-b-PAMBA in solution and the formation of self-locked nanostructures through changes in solution pH and ionic strength; and

8. Characterize the (co)polymers in terms of molecular weights, chain length distributions and functionalities using size exclusion chromatography (SEC), $^1$H and $^{13}$C NMR, UV-Vis and FT-IR spectroscopy; and the corresponding self-assembled nanostructures by static and dynamic light scattering (SLS, DLS), transmission electron microscopy (TEM) and fluorescence spectroscopy.
CHAPTER III

EXPERIMENTAL

Materials

All reagents and solvents were obtained from Sigma-Aldrich or Fisher Scientific and used as received unless otherwise stated. N,N-dimethylacrylamide (DMA, 99%, Sigma-Aldrich, St. Louis, MO, USA) and 2-(acryloyloxy)ethylisocyanate (AOI, Showa Denko K.K., Tokyo, Japan) were distilled under reduced pressure and stored below 0 °C until use. N-(2-hydroxyethyl)acrylamide (HEA, 97%, Sigma-Aldrich, St. Louis, MO, USA) was purified using inhibitor remover (Sigma-Aldrich, St. Louis, MO, USA) before polymerization. Syntheses of 3-acrylamido-3-methylbutanoic acid (AMBA) and 3-[2-(N-methylacrylamido)ethyl(dimethylammonio)propanesulfonate (MAEDAPS) were previously reported. Allylisocyanate (AI, 98%), ethanethiol (97%), 3-propanethiol (99%), carbon disulfide (99.9%), sodium hydride (60% dispersion in mineral oil), sodium thiosulfate (99%), iodine (>99%), 3-mercaptobutryonic acid (99+%), cysteamine hydrochloride, L-cysteine hydrochloride monohydrate (98+%), benzyl mercaptan (99%), thiophenol (99+%), thioglycerol (99%), mercaptosuccinic acid, 2-methyl-2-propanethiol (98%), deuterium oxide (D₂O, 99.9 atom % D), chloroform-d₉ (99.8 atom % D + 0.1 (v/v) % TMS), acetone-d₆ (99.9 atom % D + 0.1% (v/v) % TMS), methanol-d₄ (99.8 atom % D), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), dimethylphenyl phosphine (DMPP, 99%), 4,4’-methylenebis(2,6-di-tert-butylphenol) (98%), dibutyltin dilaurate (DBTDL, 95%), lectin-fluorescein isothiocyanate conjugate (FITC-Con A, from *Canavalia ensiformis*, lyophilized powder), transfer RNA (tRNA, from *Saccharomyces cerevisiae*, type X, lyophilized powder), 2-ethanolamine (99%), cyclohexylisocyanate (98%), n-propyamine (99%), pyrene (99%) and 8-anilino-1-naphthalenesulfonic acid.
(ANS, 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Triethylamine (TEA, >99.5%), 2-mercaptoethanol ( >99%) and 4,4′-azobis(4-cyanopentanoic acid) (ACPA, 99%) were obtained from Fluka (Buchs, Switzerland). 2,2′-Azobis(2,4-dimethyl valeronitrile) (ADVN, 97%) and 4,4′-azobis[2-(imidazolin-2-yl)propane] dihydrochloride (AIPD) were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan) and used as received. Deuterated dimethyl sulfoxide-d$_6$ (99.9 atom % D + 1% v/v TMS) was purchased from Cambridge Isotope Laboratories, Inc (Andover, MA, USA).

Methods and Instrumentation

NMR analyses were performed using Varian INOVA 300 or 500 MHz NMR spectrometer. For the zwitterionic copolymers, $^1$H NMR spectra (in 90/10 H$_2$O/D$_2$O mixture) were generated via manual solvent suppression technique. FT-IR spectra were recorded using modified Bruker 88 or Nicolet 8700 spectrometers. Samples were prepared by casting polymer solutions on NaCl plates. Each spectrum was collected over 32 scans with a resolution of 4.

Molecular weights and polydispersities were determined either by organic solvent or aqueous size exclusion chromatography (SEC). The former utilized 0.02 M lithium bromide in DMF as eluent at a flow rate of 1.0 mL/min. The instrument was equipped with Viscotek I-Series Mixed Bed low-MW (exclusion limit > 20K PS) and mid-MW (exclusion limit > 200K PS) columns, Viscotek triple detector array (302 nm RI, viscosity, 7 mW 90° and 7° true low angle light scattering detectors (670 nm)) equilibrated at 35 °C. Aqueous SEC system for cationic polymers utilized an eluent of 1 wt % acetic acid/0.10 M Na$_2$SO$_4$ (aq) at a flow rate of 0.25 mL/min at 25 °C, Eprogen, Inc. CATSEC columns (100, 300, and 1000 Å), a Polymer Laboratories LC1200 UV-
visible detector, a Wyatt Optilab DSP interferometric refractometer ($\lambda=690$ nm), and a Wyatt DAWN-DSP multiangle laser light scattering (MALLS) detector ($\lambda=633$ nm). The molecular weights and polydispersities of zwitterionic (co)polymers were determined using a Wyatt Technologies aqueous SEC equipped with Viscotek G4000 PW_{XL} column, LC 1200 UV-visible photometer, DAWN DSP multiple angle laser light scattering detector and Wyatt Optilab DSP interferometric refractometer. The mobile phase consisted of 80% 0.5 M NaBr and 20% acetonitrile. Flow rate was maintained at 0.5 ml min$^{-1}$ using an Agilent 1100 series pump.

Dynamic light scattering studies were conducted at 25 °C using a Malvern Instruments Zetasizer Nano series instrument equipped with a 22 mW He-Ne laser with a wavelength of 632.8 nm, an avalanche photodiode detector with high quantum efficiency, an ALV/LSE-5003 multiple tau digital correlator electronics system. Dispersion Technology Software v5.03 (Malvern Instruments Ltd) was used to record and determine the particle sizes and distributions. Static light scattering was performed using Wyatt DAWN Enhanced Optical System (DAWN$^R$ EOS$^TM$) 18-angle laser light scattering detector in batch mode. Polymer solutions were filtered using 0.2 µm nylon membrane (Millipore) directly into the light scattering cell.

Fluorescence studies were performed using Photon Technology International QuantaMaster$^TM$ fluorimeter and FeliX32$^TM$ software. Aliquots (10 µL) of 5.4 x 10$^{-4}$ M pyrene in acetone were added into vials and the solvent was allowed to evaporate. Polymer solutions (10 mL) were added into the vials to yield 0.54 µM pyrene. With ANS, 10 µL of 50 mM ANS aqueous solution was added into 10 mL polymer solutions. The concentration of the ANS probe was 50 µM.
Transmission electron microscopy (TEM) measurements were conducted with a JEOL JEM-2100 electron microscope at an accelerating voltage of 200 kV. The specimens were prepared by placing the copolymer solution (5 µL) on a carbon-coated copper grid followed by drying at ambient conditions.

**Synthesis of RAFT Chain Transfer Agents**

The RAFT chain transfer agents 2-(ethylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid (EMP), 4-cyano-4-(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid (CEP), 4-cyanopentanoic acid dithiobenzoate (CTP) and N,N-dimethyl-S-thiobenzoylthiopropionamide (TBP) were previously synthesized according to literature procedures.

![Chemical structures of RAFT chain transfer agents](image)

**Figure III-1.** Chemical structures of RAFT chain transfer agents (CTA) used to prepare the (co)polymers described in this research work.
Synthesis of 2-(ethylsulfanylthiocarbonylsulfanyl)-2,4-dimethylvaleronitrile (CEV)

CEV was synthesized following a modified literature procedure. Briefly, a slurry of sodium hydride (1.9 g, 0.08 mol) in diethyl ether (200 mL) was cooled in an ice bath for 15 minutes. To this mixture, ethanethiol (5 g, 0.080 mol) was added dropwise. The mixture was stirred for 15 minutes and carbon disulfide (6.2 g, 0.080 mol) was then added slowly forming a yellow suspension. After stirring for 1 hour, the mixture was allowed to warm to room temperature before being concentrated under reduced pressure on a rotatory evaporator. The resulting residue was suspended in diethyl ether (200 mL). Iodine (10.3 g, 0.041 mmol) was then added to convert the trithiocarbonate salt into disulfide. The solution was then washed with a sodium thiosulfate solution (2 x 200 mL, 5 wt %). The yellow organic layer was collected and dried over magnesium sulfate. After removing the drying agent, the solvent was removed under reduced pressure yielding a yellow oil. This crude product and ADVN (15.0 g, 0.060 mol) were dissolved in ethyl acetate (100 mL) and the mixture was allowed to react with stirring overnight under reflux at 70 °C. The solvent was removed under reduced pressure and the oil was purified by column chromatography. Rf = 0.40 (SiO2, ethyl acetate / hexanes, 5:95 (v/v)).

δH (300 MHz, CDCl3): 1.02-1.12 (dd, 6H, -CH(-C₃H₃)₂), 1.32-1.40 (t, 3H, -S-CH₂-CH₃), 1.78-1.86 (dd, 1H, -CH₂-CH(-CH₃)₂), 1.90-1.92 (s, 3H, -C(-CH₃)(-CN)-), 1.94-2.10 (m, 1H, -CH₂-CH(-CH₃)₂), 2.10-2.18 (dd, 1H, -CH₂-CH(-CH₃)₂), 3.30-3.40 (q, 2H, -S-C₂H₂-CH₃).

δC NMR (300 MHz, CDCl₃) δ: 12.90 (-S-CH₂-CH₃), 23.60 (-CH₂-CH(-CH₃)₂), 24.00 (-C(-CH₃)(-CN)-), 25.50 (-CH(-CH₃)₂), 25.80 (-CH(-CH₃)₂), 31.20 (-S-CH₂-CH₃), 46.50 (-S-C(-CN)(-CH₃)-), 47.30 (-CH₂-CH(-CH₃)₂), 120.00 (-CN), 217.60 (-C=S).
Scheme III-1. Synthesis of 2-(ethylsulfanylthiocarbonylsulfanyl)-2,4-dimethyl valeronitrile (CEV) following a modified literature procedure.²⁷⁵,²⁷⁹

\[
\text{SH} + \text{NaH} \xrightarrow{\text{diethyl ether}, 0^\circ \text{C}} \text{SNa}^+ \xrightarrow{\text{CS}_2} \text{SSNa}^+
\]

\[
\text{SSNa}^+ \xrightarrow{\text{I}_2} \text{SSS}
\]

\[
\text{SSS} \xrightarrow{\text{ADVN, ethyl acetate, 70}^\circ \text{C}} \text{SSCN} \xrightarrow{\text{CEV}}
\]
Figure III-2. (a) $^1$H and (b) $^{13}$C NMR spectra of 2-(ethylsulfanylthiocarbonylsulfanyl)-2,4-dimethylvaleronitrile (CEV) in CDCl$_3$. 
Polymerizations

*General Procedure for Homopolymerization of AOI (P1)*

The AOI monomer (0.41g, 2.9mmol), TBP (8.6mg, 0.034mmol) and ADVN (1.7mg, 0.0068mmol) were dissolved in anhydrous dioxane (3.2mL) in a septum-sealed vial. The solution was purged with nitrogen gas for an hour at room temperature. After purging, the vial was placed in a preheated reactor station (STEM Electrothermal RS6000) and polymerization reaction was allowed to proceed at a fixed time. For polymerization kinetics, the reaction was carried out in a rubber septum-sealed round bottom flask heated in an oil bath. Aliquots were taken from the polymerization mixture using degassed syringes at predetermined time intervals. The aliquots were cooled to room temperature and briefly exposed to the atmosphere to quench the reaction. The polymerization mixture was reacted with methanol prior to SEC analyses. To ensure that all isocyanate was completely reacted, the absorbance at 2280 cm\(^{-1}\) was monitored using FT-IR spectroscopy. The monomer conversion was determined using either UV-visible or \(^1\)H NMR spectroscopy. For UV-visible spectroscopy, 25µL of polymerization mixture was diluted with 2.5mL of tetrahydrofuran (THF). Absorbances at 250 nm due to the vinyl groups normalized to the absorbance of CTA at 303 nm were used to calculate the conversion of the AOI monomer. In the polymerization using solvents other than dioxane, UV absorbance due to the solvent significantly overlapped with that of the vinyl groups. In this case, \(^1\)H NMR spectroscopy was used to calculate monomer conversion. Briefly, 100µL of the polymerization mixture was added into 500µL of acetone-d\(_6\) and the decreases in the normalized peak areas due to the vinyl protons were correlated to the conversion of the monomer.
In the chain extension experiments, AOI monomer (0.34 g, 2.4 mmol), TBP (8.6 mg, 0.034 mmol) and ADVN (1.7 mg, 0.0069 mmol) were dissolved in anhydrous dioxane (2.5 mL). The solution was purged with N\textsubscript{2} gas for 45 minutes. Polymerization was carried in a reactor station (STEM Electrothermal RS6000) preheated at 50 °C. After 12 hours (conversion ~40 %), the reaction was quenched by cooling the mixture in an ice bath followed by brief exposure to air. Aliquots (1 mL) were taken for analysis. Into the remaining mixture, AOI (0.15 g, 1.1 mmol), ADVN (0.75 mg, 0.003 mmol) and anhydrous dioxane (1 mL) were added. The mixture was purged for 45 minutes with N\textsubscript{2} gas and allowed to react at 50 °C for an additional 8 hours. After quenching the reaction, the mixture was reacted with methanol for SEC analysis.

**Scheme III-2.** Direct RAFT polymerization of 2-(acryloyloxy)ethylisocyanate (AOI) and subsequent functionalization through reaction with alcohols, amines and thiols.

\[ R = \text{O (urethane)} \]
\[ = \text{NH (urea)} \]
\[ = \text{S (thiourea)} \]

**RAFT Polymerization of PDMA-b-PHEA (P2)**

DMA (10.0 g, 101 mmol), CEV (247 mg, 1.0 mmol) and ADVN (50.0 mg, 0.20 mmol) were dissolved in dioxane (50 mL) in a round bottom flask, equipped with a magnetic stir bar and sealed with a rubber septum. The mixture was purged with nitrogen
for 45 minutes. The polymerization was allowed to proceed at 50 °C for 18 hours
(conversion by $^1$H NMR = 95%). The PDMA$_n$-CEV macroCTA (Mn(SEC) = 10,300,
PDI = 1.04, dn/dc = 0.091) was purified by precipitation (twice) in diethyl ether (polymer
yield = 9.3 g, 98%). A mixture of HEA (11.0 g, 95.0 mmol), MEHQ inhibitor remover
and dioxane (25 mL) in a conical flask was stirred on a magnetic stir plate for 4 hours at
room temperature. After filtration, ACPA (62.0 mg, 0.22 mmol) was added to the clear
filtrate in a round bottom flask (250 mL) and allowed to dissolve with stirring. PDMA$_n$-
CEV macroCTA (8.0 g, 0.78 mmol) was dissolved in DI water (100 mL) and the
resulting solution was slowly added to the HEA/ACPA mixture. The pH of the solution
was adjusted to 5.0 using 0.1 M HCl. The mixture was then purged with nitrogen for 1.5
hours. The polymerization was carried out at 50 °C for 10 hours (conversion by $^1$H NMR
= 75%). The resulting solution was diluted with DI water and dialyzed against water at
pH 3-5 for 3 days using a dialysis membrane (Spectra/Por Regenerated Cellulose,
Spectrum Laboratories Inc, CA, USA) with MWCO of 3,500. Lyophilization yielded
PDMA$_n$-$b$-PHEA$_m$ diblock copolymer (P2). Polymer yield = 14.5 g (89%). Mn (SEC) =
23,500, PDI=1.11, dn/dc = 0.062. $\delta$H (300 MHz, DMSO-$d_6$): 0.90-2.40 (b, n x 3H, m x
3H; backbone, -CH$_2$-CH-), 2.58-3.24 (b, n x 6H, N-CH$_3$; b, m x 2H, NH-CH$_2$-), 3.39-
3.51 (b, m x 2H, -CH$_2$-O-), 4.64-5.18 (b, m x 1H, -OH), 7.19-7.96 (b, m x 1H, -NH).
Characteristic bands in FT-IR, ν (NaCl, cm$^{-1}$): 3423 (b, O-H), 3309 (b, N-H), 1643 (s,
C=O, amide), 1060 (m, C-O, hydroxyl). The full FT-IR spectrum of P2 is available in the
Appendix B.
Scheme III-3. Sequential RAFT polymerization of \(N,N\)-dimethylacrylamide (DMA) and \(N\)-(2-hydroxyethyl)acrylamide (HEA) using CEV as chain transfer agent to obtain PDMA-\(b\)-PHEA diblock copolymer, P2.

\[
\begin{align*}
\text{CEV} & \quad \text{DMA} \quad \text{HEA} \\
& \quad \text{initiator} \quad \text{initiator}
\end{align*}
\]

Scheme III-4. Aqueous RAFT synthesis of PDMA-\(b\)-PMAEDAPS-\(b\)-PAMBA triblock copolymer, P3.

RAFT Polymerization of PDMA-\(b\)-PMAEDAPS-\(b\)-PAMBA (P3)

DMA (5.0 g, 51 mmol), EMP (110 mg, 0.50 mmol) and AIPD initiator (33 mg, 0.10 mmol) were dissolved in DI water (50 ml) at 0 °C using an ice bath. The pH of the solution was adjusted to 5 to completely dissolve the CTA and initiator. The solution was purged with nitrogen for 30 minutes and polymerization was carried out at 40 °C for 5 hours. The reaction was quenched by cooling the flask in liquid nitrogen. The polymerization mixture was dialyzed against DI water for 3 days (MWCO=1000, Spectrum Laboratories Inc, CA, USA) and subsequently lyophilized to obtain the PDMA
macroCTA (Mn=10,300, PDI=1.05, dn/dc=0.16). The PDMA macroCTA (2.0 g, 0.19 mmol) was chain-extended with MAEDAPS (4.0 g, 14 mmol) using ACPA (10. mg, 0.036 mmol) as free radical source in 0.5 M NaCl solution (0.7 M monomer concentration). The solution was purged with nitrogen for 30 mins and reacted at 70 °C for 4 hours. The diblock copolymer (Mn=28,200, PDI=1.04, dn/dc=0.14) was purified by dialysis (MWCO=6-8 kDa, Spectrum Laboratories Inc, CA, USA) and dried by lyophilization. To obtain the triblock copolymer, diblock macroCTA (2.0 g, 0.071 mmol), AMBA (1.1 g, 6.4 mmol) and ACPA (4.9 mg, 0.017 mmol) were dissolved in 0.5 M NaCl solution at pH 5 (0.3 M monomer concentration). Polymerization was carried out at 70 °C for 4 hours. The polymerization mixture was dialyzed (MWCO=12-14 kDa, Spectrum Laboratories Inc, CA, USA) against DI water for 3 days and dried by lyophilization to yield the triblock copolymer (P3, Mn=40,000, PDI=1.03, dn/dc=0.14).

The chain extension polymerizations were carried out in 0.5 M NaCl aqueous solution in order to prevent precipitation of the block copolymers.

(Co)polymer Modifications

Pendent Modifications of PAOI Homopolymer

The functional agent (1.5 equivalents of alcohol with 0.1 wt% (1.6 mM) of dibutyltin dilaurate (DBTDL), 1 equivalent of amine or 1 equivalent of thiol with 0.5 wt% (50 mM) triethylamine (TEA)) were added to the solution of PAOI homopolymer (P1) containing 0.83 M of isocyanate (NCO) groups (refer to Scheme III-2). The mixture was allowed to react overnight and the complete reaction of the NCO groups was monitored by FT-IR spectroscopy. After the reaction, the mixtures were subjected to SEC to determine polymer molecular weights and polydispersities. The polymerization
mixture became decolorized, when amine and thiol agents were used, indicating the degradation of the CTA moiety as well.

Alkene Functionalization of PDMA-b-PHEA (P2-1 and P2-2)

Alkene-functionalized copolymers P2-1 and P2-2 were obtained through reaction of P2 with AOI and allylisocyanate, respectively (see Scheme III-5). Copolymer P2 (4.0 g, 14.0 mmol hydroxyl groups) and 4,4’-methylenbis(2,6-di-tert-butylphenol) (0.35 g, 0.9 wt %) were dissolved in anhydrous DMF (35 mL). An excess of isocyanate reactant (30 mmol) and DBTDL catalyst (50 mg, 0.1 wt %) were added to the polymer solution in a septum-sealed flask equipped with a magnetic stir bar. The mixture was allowed to react at 40 °C for 24-36 hours. The polymers were precipitated twice in cold diethyl ether, filtered and dried under reduced pressure for 2 hours. P2-1: yield = 4.9 g (80%); Mn(SEC) = 39,600 PDI = 1.18; δH (300 MHz, DMSO-d6): 0.90-2.40 (b, n x 3H, m x 3H; backbone, -CH2-CH-), 2.60-3.29 (b, n x 6H, N-CH3; b, m x 2H, -CH-C(O)-NH-CH2-; b, m x 2H, -O-C(O)-NH-CH2-), 3.66-4.27 (b, m x 2H, -C(O)-O-CH2-; b, m x 2H, -NH-C(O)-O-CH2-), 5.79-6.41 (multiple resonances; m x 3H; CH2=CH-), 6.80-7.87 (b, m x 2H, -NH-); Characteristic bands in FT-IR, ν (NaCl, cm⁻¹): 3305 (b, N-H), 1724 (s, C=O, carbamate, ester), 1643 (s, C=O, amide), 1253 and 1189 (s, C-O, carbamate, ester), 981 (s, vinyl). P2-2: yield = 5.3 g (94%); Mn(SEC) = 35,300 PDI = 1.14; δH (300 MHz, DMSO-d6): 0.90-2.40 (b, n x 3H, m x 3H; backbone, -CH2-CH-), 2.60-3.3 (b, n x 6H, N-CH3; b, m x 2H, C(O)-NH-CH2-), 3.48-3.74 (b, m x 2H, -O-C(O)-NH-CH2-), 3.76-4.14 (b, m x 2H, -NH-C(O)-O-CH2-), 4.93-5.23 (dd, m x 2H, CH2=CH-), 5.65-5.93 (m, m x 1H; CH2=CH-), 6.86-7.92 (b, m x 2H, -NH-); Characteristic bands in FT-IR, ν (NaCl, cm⁻¹): 3315 (b, N-H), 1714 (s, C=O, carbamate), 1643 (s, C=O, amide), 1249 and 1137 (s, C-
O, carbamate), 989 and 916 (m, vinyl). The FT-IR spectra for copolymers P2-1 and P2-2 are available in the Appendix B.

**Scheme III-5.** Post-polymerization modifications of copolymer P2 through reactions with 2-(acyloyloxy)ethylisocyanate (AOI) and allylisocyanate. Structopendent alkene-containing copolymers P2-1 and P2-2 were then utilized in Michael and free radical thiol-ene addition reactions, respectively, with selected thiols to obtain a series of functionalized copolymers.

General Procedure for Thiol Michael Addition (P2-1[a-j])

Precursor copolymer P2-1 (300 mg, 0.88 mmol ene) was dissolved in DMSO (3 mL). Into the copolymer solutions, the thiol reactant (1.3 eq SH) and amine catalyst (TEA, 0.1 eq) were added. The reaction mixtures were stirred using a magnetic stir plate at room temperature for 12 hrs. The disappearance of the vinyl resonances was confirmed using $^1$H NMR spectroscopy. After the reactions, the copolymer solutions were transferred into dialysis tubing (MWCO 6-8 kDa, Spectra/Por Regenerated Cellulose, Spectrum Laboratories Inc, CA, USA) and dialyzed against DI water with pH adjusted to
3-5 using 1.0 M HCl, changing the dialysate every 2 hours for 1 day. For hydrophobically modified copolymers, the solutions were first dialyzed against 90% (v/v) ethanol aqueous solution for 24 hrs, followed by dialysis against DI water for another 24 hrs. Dry copolymers obtained after lyophilization were characterized by SEC, NMR and FT-IR spectroscopy. The $^1$H NMR and FT-IR spectra of all the copolymers are available in the Appendix B. Molecular weights and polydispersities are shown in Table IV-3.

Copolymer yields after dialysis and lyophilization: P2-1a (300 mg, 85%), P2-1b (366 mg, >99%), P2-1c (380 mg, 99%), P2-1d (390 mg, >99%), P2-1e (340 mg, 96%), P2-1f (420 mg, >99%), P2-1g (330 mg, 88%), P2-1h (399 mg, 98%), P2-1i (350 mg, 92%), P2-1j (310 mg, 75%).

**General Procedure for Free Radical Thiol-Ene Addition (P2-2[a-j])**

Precursor copolymer P2-2 (200 mg, 0.71 mmol), thiol (10 equivalents) and free radical initiator (ADVN, 0.3 equivalent) were dissolved in DMSO (2 mL). The solutions were deoxygenated by purging with N$_2$ for 45 minutes. The solutions were then heated at 50 °C for 12 hours, followed by exposure to air to quench the reactions. The solutions were transferred to dialysis tubing (MWCO 6-8 kDa, Spectra/Por Regenerated Cellulose, Spectrum Laboratories Inc, CA, USA) and dialyzed against 90% (v/v) ethanol aqueous solution for 24 hours and then against DI water for another 24 hours, changing the dialysate every 2 hours. The solutions were then lyophilized and the purified copolymers were analyzed by SEC, NMR and FT-IR spectroscopy. The spectra of all the copolymers are available in the Appendix B. Molecular weights and polydispersities are shown in Table IV-4. Yields after dialysis and lyophilization: P2-2a (207 mg, 84%), P2-2b (182 mg, 71%), P2-2c (160 mg, 49%), P2-2d (180 mg, 73%), P2-2f (230 mg, 85%), P2-2g (209 mg, 79%), P2-2h (189 mg, 54%), P2-2i (256 mg, 93%).
Stimuli-Responsive Assembly of Copolymers

Self-Assembly of Functionalized Copolymers

The copolymers were either directly dissolved in HPLC water or aliquots of copolymer solutions in DMSO were added into HPLC water to obtain 1.0 mg mL\(^{-1}\) copolymer concentrations. The solution pH was adjusted using 0.1 M HCl or 0.1 M NaOH. The solutions were sonicated for 1 min and filtered (0.2 µm, Millipore) directly into cuvettes for DLS measurements.

Formation of “Self-Locked” Micelles

The PDMA-b-PMAEDAPS-b-PAMBA, P3, triblock copolymer was first dissolved in 0.5 M NaCl solution at pH 7 and sequentially dialyzed against: (i) 0.5 M NaCl solution at pH 7 (1 day) to ensure complete dissolution, (ii) 0.5 M NaCl solution at pH 4 (1 day) to protonate the AMBA block and promote self-assembly, (iii) decreasing NaCl concentrations in pH 4 solutions (3 days) and (iv) DI water with pH adjusted to 4 (3 days) to finally obtain the “self-locked” micelles. The stimuli-triggered micelle formation was investigated using dynamic and static light scattering (DLS/SLS), \(^1\)H NMR spectroscopy, fluorescence spectroscopy and transmission electron microscopy TEM.

For fluorescence studies, aliquots (10 µL) of 5.4 x 10\(^{-4}\) M pyrene in acetone were added into vials and the solvent was allowed to evaporate. Polymer solution (10 mL) at pH 7 was added into the vials to yield 1.0 wt % polymer concentration. With ANS, 10 µL of 50 mM ANS aqueous solution was added into 10 mL of 1.0 wt % polymer solution at pH 7. After 5 hours, the pH of the solution was slowly adjusted to 4 with 0.1 M HCl. The solutions were left at room temperature overnight before measurement. The concentrations of the probe were 0.54 µM and 50 µM for pyrene and ANS, respectively.
CHAPTER IV
RESULTS AND DISCUSSION

This chapter is divided into three sections. In Section I, the direct RAFT homopolymerization of 2-(acyrloyloxy)ethylisocyanate (AOI) and subsequent post-polymerization modifications are described. The reactions between the pendent isocyanate groups and small molecule compounds having amine, alcohol or thiol groups are a facile means to functionalizing PAOI homopolymer. A related strategy is described in Section II. A hydroxyl-containing diblock copolymer precursor was first prepared via RAFT polymerization. The pendent hydroxyl groups were reacted with bifunctional alkene-isocyanate linkers yielding alkene-functionalized copolymer precursors. Selected thiols having alkyl, aryl, hydroxyl, carboxylic acid and amine functionalities were then conjugated to the structopendent alkenes via Michael-type as well as free radically mediated thiol-ene addition reactions. Utilizing two sequential reactions, a library of functional copolymers was generated from a single copolymer precursor. The synthesis and solution studies of pH- and salt-responsive triblock copolymer are detailed in Section III. This system is capable of forming self-locked micellar structures which may be controlled by changing solution pH as well as ionic strength.
Section I. Direct RAFT Polymerization of an Unprotected Isocyanate-Containing Monomer and Subsequent Structopendant Functionalization Using Click-Type Reactions

Overview

Applications of (co)polymers in areas including nanomedicine, biotechnology, and electronics require macromolecules with controllable structures and compositions. Thus considerable effort has been devoted over the past two decades toward rational design of polymer architectures with specific physico-chemical characteristics.\textsuperscript{45,120} Recently, there has been increased activity in this arena, primarily due to the establishment of the free radical polymerization techniques that allow for the synthesis of (co)polymers that have controlled molecular weights and narrow molecular weight distributions.\textsuperscript{19} Additionally, extensive work has been dedicated toward preparing polymer “scaffolds” with reactive functionality capable of further, highly efficient reactions.\textsuperscript{4} The direct homopolymerization of monomers with structopendent reactive groups assures that each repeating unit possesses the desired functionality. However, functional groups such as free thiols and amines which can act as catalyst deactivators or chain transfer agents are only rarely tolerated by the usual polymerization methods. While protection-deprotection chemistries have been utilized, these approaches often require multiple synthetic and purification steps and are often less efficient. Given these limitations, an attractive route to post-polymerization derivatization is the combination of direct, controlled free radical polymerization (CRP) yielding reactive pendent functionality followed by highly specific and efficient “click”-type reactions.\textsuperscript{69}

It is well known that the reactions between isocyanates and active hydrogen containing functional groups (\textit{i.e.} alcohols, thiols, and amines) are efficient and quantitative under certain reaction conditions. In contrast to reaction with alcohols,
isocyanates react with amines and thiols (in the presence of a base catalyst) rapidly in an efficient often selective manner.\textsuperscript{69, 117, 281-286} As such these reactions exhibit many of the attributes described for “click” chemistry.\textsuperscript{69, 287} Base-catalyzed reactions of thiols and isocyanates have recently been utilized in the chain end functionalization of thiol-terminated polymers prepared by RAFT.\textsuperscript{206} While the reactions of isocyanates and alcohols are not always fast and efficient, these can be quantitative depending on the reaction conditions. Reports of free radical polymerization of isocyanate-bearing monomers are limited.\textsuperscript{288-294} Barner et al. reported surface grafting of styrene and \textit{m}-isopropenyl-\textit{α,α’}-dimethylbenzylisocyanate onto a polypropylene solid support \textit{via} γ-initiated RAFT polymerization.\textsuperscript{292} Statistical RAFT copolymers containing a limited number of isocyanate pendant groups for efficient crosslinking were very recently reported in a collaborative effort between the Hawker and Wooley groups.\textsuperscript{294} The copolymers, which were statistical copolymers of 2-(methacryloyloxy)ethylisocyanate (5-20%) and methyl methacrylate, were reacted with diamines in dilute solutions to afford intramolecularly cross-linked nanoparticles.

The objectives of the project described in this section were: (a) to develop a direct procedure for controlled free radical polymerization of an isocyanate-containing monomer, in this case 2-(acryloyloxy)ethylisocyanate (AOI), without the need of protecting groups; (b) to determine the reaction parameters including reagent stoichiometry and solvent yielding the best control over molecular weight, polydispersity, and maintenance of pendant isocyanate functionality; and (c) to demonstrate facile side-chain reactions exhibiting the attributes of “click” type chemistry utilizing model amine, alcohol, and thiol compounds. Under anhydrous conditions, moderate temperature and by judicious choice of stoichiometry, CTA, and solvent, reasonable control of reaction
kinetics, molecular weight, and polydispersity can be attained in the RAFT polymerization of 2-(acryloyloxy)ethylisocyanate. This is the first literature study of homopolymerization of an isocyanate-based monomer by a CRP technique. Additionally, facile post-polymerization modifications with model amines, alcohols and thiols, as well as selective reaction pathways for addition of the difunctional ethanolamine and mercaptoethanol are demonstrated.

RAFT Polymerization

Initial screening experiments in preparing homopolymers of AOI for further reaction, as illustrated in Scheme III-2, involved RAFT polymerization utilizing a number of dithioester and trithiocarbonate CTAs at temperatures in the 40 to 60 °C range. Trithiocarbonates EMP and CEP as well as the dithioester CTP yielded polymers with broad molecular weight distributions while TBP was successful in producing narrow molecular weight distributions. Examples of SEC traces from the polymerization of AOI using these four CTAs are shown in Figure IV-1. These four chain transfer agents have been previously utilized in the RAFT polymerization of (meth)acrylamides, (meth)acrylates and styrenics. However, it is apparent that the carboxylic acid functionality present in the first three interfere with controlled polymerization. Carboxylic acid groups catalyze or directly react with isocyanates, the latter usually occurring at temperatures higher than the 50-60 °C utilized in our initial experiments. Nonetheless, possible coupling of a few CTA carboxylic acid groups with pendent isocyanate groups could easily explain the broadening of the molecular weight distributions. Quite good control was attained utilizing TBP; therefore, it was chosen in further experiments for optimization of polymerization conditions.
Figure IV-1. SEC traces from the RAFT polymerization of AOI using EMP, CEP, CTP and TBP as chain transfer agents in dioxane at 50 °C (Monomer/CTA/Initiator 67:1:0.2). The polymers were reacted with methanol prior to SEC analysis.

Table IV-1 shows data from the RAFT polymerization of AOI under selected reaction conditions. The possibility of side reactions might be expected at elevated temperatures, and hence, polymerizations were conducted at temperatures ranging from 40-60 °C. For a fixed amount of initiator, increasing polymerization temperature also increases the rate of radical generation which, in effect, favors radical-radical coupling reactions that can eventually alter the control of the RAFT process. To have good control in RAFT polymerization, a delicate balance between the rate of radical generation, monomer addition (i.e., propagation) and termination reactions must be maintained.\textsuperscript{44, 295} In addition, there must be a constant radical flux to sustain the polymerization to completion.\textsuperscript{54} For fixed CTA to initiator ratios, polydispersities of PAOI increase with temperature (Table IV-1 Entries 1 and 3) and for a fixed temperature, polydispersities increase with decreasing CTA to initiator ratios (i.e., increasing the amount of initiator).
(Table IV-1 Entries 1-7). Very low monomer conversion was observed with polymerization at 40 °C (Table IV-1 Entry 11) which is attributed to the low rate of initiator decomposition at 40 °C. These results indicate that the polymerization temperature has an effect on the control of the RAFT homopolymerization of AOI monomer using TBP as the CTA and the best control was achieved for polymerizations at 50 °C.

RAFT polymerization can be conducted in a wide range of reaction media including protic and aprotic organic solvents, water, and even in less common systems such as ionic liquids and supercritical carbon dioxide. Owing to the highly reactive pendent isocyanate groups, RAFT polymerizations in selected solvents were also performed. The lowest polydispersities were obtained with acetonitrile and dioxane; however, very low conversion was attained with the former. The more polar solvents (NMP and DMF) yielded slightly broader polydispersities.
Table IV-1. Direct RAFT Polymerization of 2-(Acryloyloxy)ethylisocyanate (AOI) in Dioxane. Experiments Were Conducted Using TBP as the Chain Transfer Agent and ADVN as the Free Radical Initiator. Monomer Concentration Was 0.9 M.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temperature</th>
<th>Monomer/CTA/Initiator</th>
<th>Solvent</th>
<th>Time (hr)</th>
<th>Conversion (%)</th>
<th>Mn (theo)$^a$</th>
<th>Mn (exptl)$^b$</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60 °C</td>
<td>67:1:0.2</td>
<td>Dioxane</td>
<td>12</td>
<td>64</td>
<td>7,671</td>
<td>9,600</td>
<td>1.72</td>
</tr>
<tr>
<td>2</td>
<td>60 °C</td>
<td>67:1:1.0</td>
<td>Dioxane</td>
<td>12</td>
<td>70</td>
<td>8,367</td>
<td>8,800</td>
<td>2.26</td>
</tr>
<tr>
<td>3</td>
<td>50 °C</td>
<td>74:1:0.2</td>
<td>Dioxane</td>
<td>13</td>
<td>40</td>
<td>5,341</td>
<td>9,300</td>
<td>1.13</td>
</tr>
<tr>
<td>4</td>
<td>50 °C</td>
<td>67:1:0.3</td>
<td>Dioxane</td>
<td>12</td>
<td>33</td>
<td>4,078</td>
<td>9,200</td>
<td>1.30</td>
</tr>
<tr>
<td>5</td>
<td>50 °C</td>
<td>276:1:0.2</td>
<td>Dioxane</td>
<td>15</td>
<td>59</td>
<td>28,424</td>
<td>36,000</td>
<td>1.31</td>
</tr>
<tr>
<td>6</td>
<td>50 °C</td>
<td>276:1:0.3</td>
<td>Dioxane</td>
<td>15</td>
<td>58</td>
<td>27,947</td>
<td>34,600</td>
<td>1.41</td>
</tr>
<tr>
<td>7</td>
<td>50 °C</td>
<td>276:1:1.0</td>
<td>Dioxane</td>
<td>15</td>
<td>72</td>
<td>34,632</td>
<td>33,800</td>
<td>1.63</td>
</tr>
<tr>
<td>8</td>
<td>50 °C</td>
<td>79:1:0.2</td>
<td>NMP</td>
<td>13</td>
<td>45</td>
<td>6,435</td>
<td>7,300</td>
<td>1.35</td>
</tr>
<tr>
<td>9</td>
<td>50 °C</td>
<td>79:1:0.2</td>
<td>DMF</td>
<td>13</td>
<td>45</td>
<td>6,435</td>
<td>10,200</td>
<td>1.31</td>
</tr>
<tr>
<td>10</td>
<td>50 °C</td>
<td>82:1:0.2</td>
<td>Acetonitrile</td>
<td>12</td>
<td>20</td>
<td>3,102</td>
<td>7,900</td>
<td>1.09</td>
</tr>
<tr>
<td>11</td>
<td>40 °C</td>
<td>67:1:0.3</td>
<td>Dioxane</td>
<td>12</td>
<td>&lt;3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ These values were calculated using methyl carbamate side chain.

$^b$ These values were determined from SEC after reacting the polymerization mixture with methanol.
**Figure IV-2.** (A) SEC chromatograms (RI detector), (B) kinetic plots and (C) molecular weight and PDI as a function of monomer conversion for AOI RAFT polymerization using TBP in dioxane at 50 °C (3:1 CTA to initiator ratio). Aliquots were reacted with methanol prior SEC analysis.

**Kinetics of RAFT Polymerization**

Polymerization kinetic experiments were conducted to determine if the control of RAFT polymerization varies with monomer conversion. The kinetic profile of AOI polymerization in dioxane at 50 °C was investigated using TBP and ADVN as CTA and free radical initiator, respectively. As can be ascertained from the resulting
chromatograms shown in Figure IV-2A, the evolution of molecular weight with conversion was well controlled. In addition, the respective chromatographic traces from successive aliquots in the polymerization mixture at predetermined time intervals shifted well towards lower elution volume with conversion (or polymerization time). The corresponding kinetic plot is shown in Figure IV-2B. The polymerization at 50 °C is slow, reaching only about 35% monomer conversion after 15 hours. The rate of polymerization decreases slightly at higher conversion as indicated by the changes in slope ($k_p[P\cdot]$) of the kinetic plot. This behavior is often observed for RAFT polymerization mediated by dithioester-based chain transfer agents which can require relatively long polymerization times.\textsuperscript{304-306}

The molecular weights increase linearly with conversion; however, in the later stages of the reaction, the experimental molecular weights deviate from the theoretical values (refer to Figure IV-2C). With the polymerization at 50 °C, a positive deviation in the molecular weights was observed at longer polymerization times. This molecular weight overshoot is likely due to CTA loss and/or irreversible termination reactions of the intermediate radicals.\textsuperscript{304, 305} For the synthesis of higher molecular weight polymers (requiring higher [AOI]/[CTA]) and with longer polymerization times, broader PDI values were observed (see Table IV-1 Entries 5-7). Based on these results, the polymerization of AOI monomer using TBP as the RAFT chain transfer agent appears to be best controlled at 50 °C using dioxane as the reaction medium and allowing the reaction to proceed to moderate conversions.
**Figure IV-3.** SEC chromatograms from the chain extension (self-blocking) of poly(2-(acyryloyloxy)ethylisocyanate) (PAOI).

*Chain Extension of PAOI MacroCTA*

In chain extension or block copolymer formation utilizing the RAFT technique, it is important to choose reaction conversions at which primary chain coupling and thus loss of thiocarbonylthio groups is minimal. PAOI macroCTA (Mn=6,600 PDI=1.17) was prepared by quenching the reaction at ~40% monomer conversion. The reaction was charged with more monomer, free radical initiator and solvent and the polymerization was restarted to produce chain extended polymer. The chromatograms before and after chain extension are shown in Figure IV-3. The chain extended polymer has a slightly higher polydispersity. The absence of low molecular weight shoulder or significant tailing indicates the all the chains contained the CTA moiety and had been successfully chain extended (self-blocking).
Scheme IV-1. Side-chain functionalization of poly(2-(acryloyloxy)ethylisocyanate) (PAOI) with monofunctional alcohol, thiol and amine.

Side-Chain Functionalization

The pendent isocyanate groups can be reacted with compounds containing active hydrogens such as alcohols, amines and thiols under appropriate conditions making PAOI a versatile polymer backbone for side chain conjugation and functionalization. Urea formation from the reaction of amines and isocyanates is one of the “click” reactions previously described in literature. Recently, two reports have demonstrated that the base-catalyzed reactions of isocyanates with thiols also exhibit the characteristics of “click” reactions and suggested that they be considered as such. With this in mind, the efficiency and selectivity of such reactions by performing model studies using cyclohexylisocyanate (CHI) and small molecule amines, thiols and alcohols were investigated. Details can be found in the Appendix A of this dissertation. The reactions of amines and thiols (in the presence of a basic catalyst) with isocyanate are fast and efficient. By contrast, the reactions of isocyanates and alcohols are relatively slower but can proceed to completion with the added dibutyltin dilaurate (DBTDL) as a catalyst. Surprisingly however, the formation of thiourethane groups, (especially in the absence of or at low levels of catalyst) when the difunctional mercaptoethanol was used instead of ethanol, was also observed.
The reactions of the structopendent isocyanate group of PAOI homopolymers with agents containing hydroxyl, amine and thiol groups (see Scheme IV-1) were investigated to demonstrate utility in the synthesis of functional polymers. Without further purification, the polymerization mixture containing PAOI homopolymer (0.83 M NCO) was reacted with hexanol, hexylamine and hexanethiol. DBTDL (0.1 wt%, 1.6 mM) was added as catalyst for the reaction with alcohol. In the reaction with hexanethiol, triethylamine (TEA) (0.5 wt%, 50 mM) was utilized as a base. The base deprotonates the thiol generating a thiolate anion which reacts rapidly with the isocyanate. The reaction of isocyanate with primary or secondary amines does not require catalysis. The completeness of the reaction was followed by FT-IR spectroscopy. Figure IV-4 shows the disappearance of the isocyanate peak at 2280 cm\(^{-1}\) after the reactions. The SEC traces for PAOI reaction products with hexanol, hexylamine and hexanethiol are shown in Figure IV-5. The SEC traces are unimodal and the corresponding PDI values remain low. Additionally, the formation of respective urea (NCO + amine), urethane (NCO + OH) and thiourethane (NCO + SH) bonds was confirmed by inspection of the carbonyl region of the FT-IR spectra of the products (see Figure IV-6A). The carbonyl absorbances for urea, urethane and thiourethane are centered at 1670 cm\(^{-1}\), 1724 cm\(^{-1}\) and 1680 cm\(^{-1}\), respectively. These positions are in accordance with a previous report in literature.\(^{117}\) The urethane peak at 1724 cm\(^{-1}\) overlaps the ester peak of the AOI repeating unit.
Figure IV-4. FT-IR spectra showing the disappearance of the NCO absorbance after the reaction of PAOI with alcohols, amines or thiols.

Figure IV-5. SEC chromatograms of PAOI after reaction with hexylamine (Mn=12,300 PDI=1.16), hexanol with 0.1 wt % DBTDL (Mn=9,100 PDI=1.23), and hexanethiol with 0.3 wt% TEA (Mn=12,200 PDI=1.26).
Figure IV-6. FT-IR spectra showing the carbonyl region of PAOI after reaction with (A) monofunctional and (B) difunctional agents.
As described previously, the reactions of isocyanate with amines and thiols (in the presence of a base catalyst) are fast and efficient while those of alcohols are relatively slower but can be made quantitative under certain reaction conditions. Herein, the selectivity of the reaction between isocyanate/amine and isocyanate/alcohol and between isocyanate/thiol and isocyanate/alcohol are illustrated using ethanolamine and mercaptoethanol, respectively (Scheme IV-2). The reaction of the amine functionality and isocyanate (NCO:NH$_2$:OH=1:1:1) is faster than that of alcohol group as demonstrated in the case of ethanolamine and the reaction does not require a catalyst. The isocyanate/alcohol reaction could not be made selective over the isocyanate/amine reaction since primary or secondary amines are much more reactive than alcohols. With the stoichiometric amounts of the reacting groups (NCO:SH:OH=1:1:1), the base-catalyzed reaction of thiol and isocyanate is extremely fast such that the NCO group is exclusively converted into a thiourethane. By contrast to the reaction with hexanol discussed previously, the isocyanate/alcohol reaction using mercaptoethanol (catalyzed by DBTDL) resulted in competitive formation of urethane and thiourethane linkages. The thiourethane formation, however, could be minimized by increasing the concentration of DBTDL catalyst. From previous literature reports and from our model studies, thiols are relatively unreactive towards isocyanates under neutral conditions and even in the presence of DBTDL catalyst. This is not surprising as free thiols are less nucleophilic compared to hydroxyl groups. Once deprotonated, however, the thiolate anion becomes an excellent nucleophile and reacts immediately with isocyanate (refer to the Appendix A for experimental results and discussion of the model reactions).
Scheme IV-2. Side-chain functionalization of poly(2-(acryloyloxy)ethylisocyanate) (PAOI) with 2-ethanolamine and 2-mercaptoethanol.

The FT-IR spectra of the carbonyl region of the PAOI after reaction with ethanolamine and mercaptoethanol are illustrated in Figure IV-6B. Successful side-chain functionalization was observed based on the characteristic bands for urea, urethane and thiourethane groups. However, the presence of small shoulder peak due to the thiourethane in the reaction of PAOI and mercaptoethanol catalyzed by DBTDL was also
observed. The corresponding SEC chromatograms of the PAOI homopolymers after the reaction with ethanolamine and mercaptoethanol are shown in Figure IV-7. The resulting homopolymer from the reaction of PAOI with ethanolamine exhibited good polydispersity and was soluble in water. With mercaptoethanol and TEA, the polydispersity was also low but the functionalized homopolymer was not completely soluble in water. A very broad distribution, however, was obtained from the reaction of PAOI and mercaptoethanol with added DBTDL. Initially, disulfide bridges formed from oxidation of the pendant thiol groups was speculated to have caused this broad polydispersity. Attempts to break the linkages by adding reducing agents including dithiothreitol and TCEP were unsuccessful. From the model reactions, this increase in polydispersity was postulated to be due to the reaction of both the alcohol and thiol groups in mercaptoethanol when DBTDL is used as the catalyst. In the initial experiments, 0.01 wt % DBTDL was utilized and a functionalized polymer with a PDI of 1.58 was obtained (see Figure IV-7). When the amount of DBTDL catalyst was increased to 0.1 wt% and a better polydispersity was achieved. However, it was still higher as compared to the homopolymers functionalized with ethanolamine and mercaptoethanol utilizing TEA as the catalyst. While thiourethane formation in reactions using small molecules was minimized or possibly prevented with 0.1 wt% DBTDL catalyst, this was not completely avoided in the reaction with PAOI homopolymers. Lastly, it should be noted that gelation was not observed in the reaction of PAOI and mercaptoethanol utilizing this catalyst.
Figure IV-7. SEC chromatograms of PAOI after reaction with ethanolamine and mercaptoethanol. In the reaction with mercaptoethanol, (A) 0.01 wt % and (B) 0.1 wt % of DBTDL catalyst were used. (A) and (B) are from different polymerizations and have different degrees of polymerization. Their reactions with 2-ethanolamine and 2-mercaptoethanol are shown for comparison. The following are the corresponding MW and PDI values for (A) with ethanolamine (Mn=12,600 PDI=1.26), with mercaptoethanol and 0.01 wt % DBTDL (Mn=47,800 PDI=1.58), and with mercaptoethanol and 0.5 wt % TEA (Mn=14,900 PDI=1.20); and for (B) with ethanolamine (Mn=20,500 PDI=1.24), with mercaptoethanol and 0.1 wt % DBTDL (Mn=28,900 PDI=1.46), and with mercaptoethanol and 0.5 wt% TEA (Mn=23,200 PDI=1.23).
Section II. Structopendent Transformations of RAFT Block Copolymers via Sequential Isocyanate and Thiol-Ene Reactions

Overview

Over the past decade, controlled polymerization (CP) methods and click-type chemical reactions have provided unprecedented opportunities for rational design and synthesis of materials particularly useful in the fields of personal care, water purification, and nanomedicine.\(^3, 6, 57, 85, 120\) Recent advances in CP have allowed for the direct synthesis of polymers containing a wide array of functional groups with remarkable molecular weight control. However, in order to attain targeted properties, efficient post-polymerization transformation strategies are often required. The click chemistry concept, as described by Sharpless and colleagues,\(^69\) and related strategies have provided attractive routes for the modification of structoterminal and structopendent groups of polymeric precursors.\(^4, 86, 109\)

There are a number of chemical reactions that meet the requisite features of click chemistry, with the copper(I)-mediated azide-alkyne 1,3-dipolar cycloaddition being the most recognized.\(^6\) Another resurgent technique, the well-established addition of thiols to unsaturated carbon-carbon bonds, is now being referred to as thiol-ene click chemistry.\(^110-113, 141, 142, 155\) Thiol-ene addition reactions are especially attractive since they occur in a facile manner under mild conditions and do not require metal catalysts.\(^86\) In addition, thiol-containing proteins, glycoproteins and other bio-relevant species can be conjugated easily to synthetic scaffolds, often without requiring protecting group chemistry.\(^109\) Thiol-ene click chemistry enables a modular approach for attaching diverse functionalities onto the polymer chain and thus tuning of chemical and physical properties. Several examples are reported in the literature that involve conversion of the
terminal thiocarbonylthio functionality of polymers prepared via reversible addition-fragmentation chain transfer (RAFT) polymerization to the thiol groups for subsequent thiol-ene click reactions. Likewise, pendent group transformations via thiol-ene click chemistry are also powerful synthetic tools for altering (co)polymer structure.

A review of the literature reveals a number of attempts to prepare linear polymers with pendent alkenes. For example, the selective polymerization of asymmetric bifunctional vinyl monomers using controlled radical polymerization techniques has been reported. These techniques are successful only when the reactivity of the pendent alkene is sufficiently different from that of the alkene incorporated into the backbone. Many attempts to directly polymerize asymmetric bifunctional vinyl monomers have resulted in broad molecular weight distributions and formation of branched or cross-linked structures. Alternatively, well-defined polymer precursors with pendent alkenes may be prepared by ring-opening and/or ionic polymerizations of appropriate monomers. However, these polymerization methods typically employ metal catalysts, require stringent reaction conditions and are less tolerant of functional groups and impurities. Thus, more versatile synthetic routes for preparing structopendent alkene-containing polymeric precursors are needed.

Herein, a synthetic protocol that utilizes RAFT polymerization and sequential reactions involving carbamate (urethane) linkage formation and thiol-ene click addition for the syntheses of well-defined, functional block copolymers is described. First, a hydroxyl-containing diblock copolymer precursor was prepared via RAFT polymerization. Pendent alkene functional groups were then obtained by reacting the precursor diblock copolymer with isocyanates having either acrylate or allyl groups.
Since these *in situ* reactions do not generate by-products, extensive purification steps are eliminated. Thiol-ene addition reactions were then carried out using selected small molecule thiols having alkyl, aryl, hydroxyl, amine, carboxylic acid and amino acid functionalities to demonstrate the utility of the procedure (see Scheme III-5). The combination of RAFT polymerization, efficient carbamate formation and subsequent thiol-ene click addition thus provides a facile route for preparing functional copolymers for applications that require precise control over polymer architectures.

*RAFT Polymerization*

The precursor diblock copolymer P2 poly[(N,N-dimethylacrylamide)-b-(N-(2-hydroxyethyl) acrylamide)] (PDMA$_n$-b-PHEA$_m$, Mn(SEC) = 23,500 PDI=1.11) was synthesized directly (in the absence of protecting groups) by sequential RAFT polymerization of DMA and HEA monomers using 2-(ethylsulfanylthiocarbonyl sulfanyl)-2,4-dimethylvaleronitrile (CEV) as the chain transfer agent (CTA). CEV was utilized in the RAFT polymerization as it affords excellent control and produces polymers with termini that do not interfere in the reaction with isocyanates. The SEC traces and molecular weights of PDMA$_n$-CEV macroCTA and PDMA$_n$-b-PHEA$_m$ diblock copolymer (P2) are shown in Figure IV-8 and Table IV-2, respectively.

*Alkene Functionalization*

The hydroxyl groups of the HEA block were reacted in separate reactions with two alkene-containing isocyanates, 2-(acryloyloxy)ethyl isocyanate (AOI) and allylisocyanate (AI), in the presence of dibutyltin dilaurate (DBTDL) catalyst resulting in acrylate- (P2-1) and allyl-functionalized (P2-2) precursor copolymers, respectively. These reactions were conducted at 40 °C in the presence of 4,4’-methylenebis (2,6-di-tert-butylphenol) inhibitor to prevent the alkene groups from polymerizing. $^1$H NMR
spectra of \textbf{P2, P2-1} and \textbf{P2-2} are shown in Figure IV-9. The quantitative conversions were confirmed by the following: (a) complete disappearance of the hydroxyl group resonances at 4.64-5.18 ppm, (b) shift of neighboring methylene proton resonances from 3.39-3.51 to 3.7-4.2 ppm, (c) appearance of resonances due to vinylic protons at 5.7-6.5 for acrylate and 5-6 ppm for allyl groups, and (d) the appearance of the carbamate N-H signal at 7.20-7.40 ppm. The consumption of the hydroxyl groups was also qualitatively observed by the changes in FT-IR spectra of \textbf{P2, P2-1} and \textbf{P2-2} (spectra are available in the Appendix B). In addition to the change in shape of the broad band at 3600-3200 \text{cm}^{-1} attributed to the reaction of hydroxyl groups, copolymers \textbf{P2-1} and \textbf{P2-2} also showed vibrations at 1724 \text{cm}^{-1} from carbamate and ester carbonyl groups, C-O stretching vibrations at 1250 and 1150 \text{cm}^{-1} and characteristic vibrations of the alkene moieties between 1000-900 \text{cm}^{-1}. Lastly, the success of the coupling reaction was indicated by the increase in molecular weight of copolymers \textbf{P2-1} and \textbf{P2-2} as compared to its precursor copolymer \textbf{P2} (see Figure IV-8, Table IV-2). The experimental Mn values agree with the theoretically predicted molecular weights.
Figure IV-8. SEC traces (RI) of PDMA\textsubscript{n} macroCTA, PDMA\textsubscript{n}-b-PHEA\textsubscript{m} diblock copolymer (P2) and the resulting alkene-functionalized copolymers P2-1 and P2-2. Polymer molecular weights and polydispersities are shown in Table IV-2.
Table IV-2. Molecular Weights And Polydispersities of PDMAₙ MacroCTA, PDMAₙ-b-PHEAₙ Diblock Copolymer, PDMAₙ-b-PHEA(acrylate)ₘ and PDMAₙ-b-PHEA(allyl)ₘ Precursor Copolymers.

<table>
<thead>
<tr>
<th>(Co)polymer a</th>
<th>Mn(theo)</th>
<th>Mn(NMR)</th>
<th>Mn(SEC) b</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMA₉₆</td>
<td>9,800</td>
<td>9,500</td>
<td>10,300</td>
<td>1.04</td>
</tr>
<tr>
<td>P2 PDMA₉₆-b-PHEA₁₁₅</td>
<td>23,100</td>
<td>19,000</td>
<td>23,500</td>
<td>1.11</td>
</tr>
<tr>
<td>P2-1 PDMA₉₆-b-PHEA(acrylate)₁₁₅</td>
<td>39,300</td>
<td>30,600</td>
<td>39,600</td>
<td>1.18</td>
</tr>
<tr>
<td>P2-2 PDMA₉₆-b-PHEA(allyl)₁₁₅</td>
<td>32,600</td>
<td>25,800</td>
<td>35,300</td>
<td>1.14</td>
</tr>
</tbody>
</table>

a Degrees of polymerization (DP) were calculated using theoretical molecular weights.
b Determined using SEC (DMF).
Figure IV-9. $^1$H NMR spectra (in DMSO-$d_6$) of (a) PDMA$_n$-b-PHEA$_m$ (P2) diblock copolymer and alkene-functionalized precursor copolymers (b) P2-1 and (c) P2-2. Efficiencies of functionalization were determined from the disappearance and appearance of characteristic resonances due to the reactions of alkene isocyanate linkers (i.e. 2-(acyloyloxy)ethylisocyanate, AOI, and allylisocyanate) with the pendent hydroxyl groups.
**Thiol-Ene Click Addition**

Hydrothiolation of alkenes can be generally categorized as anionic Michael-type additions or as free radically-mediated reactions. Driven by the high nucleophilicity of the thiolate anion, the former is effective with electron-deficient alkenes (e.g., (meth)acrylates, (meth)acrylamides, maleimides, etc). However, the free radical type addition of thiols to electron deficient alkenes is not as effective due to inherently low reaction rates and competing side reactions such as homopolymerization. With these considerations in mind, precursor copolymers P2-1 and P2-2 were modified via Michael and free radical thiol-ene addition reactions, respectively, using selected thiols (see Scheme III-5).

Amines are effective catalysts for thiol Michael-type addition. In the base-catalyzed reaction, a proton is abstracted from the thiol forming a thiolate anion and the conjugate acid. The thiolate anion then adds to the less hindered beta carbon of the alkene and generates the carbon-centered anion (enolate) intermediate that immediately abstracts a proton from a donor (i.e., the conjugate acid or a thiol), yielding the thiol Michael addition product and regenerating the base or thiol anion. In the free radical-mediated thiol-ene addition, the free radical may be generated photochemically or thermally using appropriate initiator. The generated free radical first abstracts a hydrogen atom from the thiol. The formed thyl free radical then adds to the carbon-carbon double bond in anti-Markownikov fashion. The carbon-centered free radical subsequently abstracts a hydrogen atom from another thiol, regenerating the thyl free radical. This chain reaction continues until all the reactants are consumed.
Table IV-3. Copolymers Prepared Via Michael-type Addition of Thiols to Precursor Copolymer P2-1 Using Triethylamine (TEA) as Catalyst and DMSO as Solvent (100 mg mL⁻¹ Copolymer Concentration, 12 hrs, 20 °C). Efficiency of Conjugation Was Measured by \(^1\)H NMR Spectroscopy.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Thiol</th>
<th>Ene : Thiol : Catalyst</th>
<th>Conversion %</th>
<th>Mn(theo)</th>
<th>Mn(SEC)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2-1a</td>
<td><img src="image1" alt="SH" /></td>
<td>1.0 : 1.3 : 0.1</td>
<td>&gt;99</td>
<td>48,000</td>
<td>50,600</td>
<td>1.18</td>
</tr>
<tr>
<td>P2-1b</td>
<td><img src="image2" alt="SH" /></td>
<td>1.0 : 5.0 : 0.1</td>
<td>&gt;99 (83)⁺</td>
<td>49,700</td>
<td>52,400</td>
<td>1.17</td>
</tr>
<tr>
<td>P2-1c</td>
<td><img src="image3" alt="SH" /></td>
<td>1.0 : 1.3 : 0.1</td>
<td>&gt;99</td>
<td>53,600</td>
<td>74,200</td>
<td>1.14</td>
</tr>
<tr>
<td>P2-1d</td>
<td><img src="image4" alt="SH" /></td>
<td>1.0 : 1.3 : 0.1</td>
<td>&gt;99</td>
<td>52,000</td>
<td>71,500</td>
<td>1.17</td>
</tr>
<tr>
<td>P2-1e</td>
<td><img src="image5" alt="SH" /></td>
<td>1.0 : 1.3 : 0.1</td>
<td>&gt;99</td>
<td>48,300</td>
<td>59,400</td>
<td>1.18</td>
</tr>
<tr>
<td>P2-1f</td>
<td><img src="image6" alt="SH" /></td>
<td>1.0 : 1.3 : 0.1</td>
<td>&gt;99</td>
<td>51,700</td>
<td>48,600</td>
<td>1.02</td>
</tr>
<tr>
<td>P2-1g</td>
<td><img src="image7" alt="SH" /></td>
<td>1.0 : 1.3 : 1.1</td>
<td>&gt;99</td>
<td>51,500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P2-1h</td>
<td><img src="image8" alt="SH" /></td>
<td>1.0 : 1.3 : 2.1</td>
<td>&gt;99</td>
<td>56,600</td>
<td>44,300</td>
<td>1.18</td>
</tr>
<tr>
<td>P2-1i</td>
<td><img src="image9" alt="SH" /></td>
<td>1.0 : 1.3 : 1.1</td>
<td>&gt;99</td>
<td>52,400</td>
<td>42,900</td>
<td>1.02</td>
</tr>
<tr>
<td>P2-1j</td>
<td><img src="image10" alt="SH" /></td>
<td>1.0 : 1.3 : 2.1</td>
<td>&gt;99</td>
<td>57,400</td>
<td>52,400</td>
<td>1.01</td>
</tr>
</tbody>
</table>

⁺Use of 1.3 equivalents of thiol yielded 83% conjugation.

⁻Determined using SEC (DMF).

⁺⁺Determined using aqueous SEC.
The thiol conjugations to copolymer P2-1 via Michael-type addition were carried out in DMSO using triethylamine (TEA) as the base catalyst. In the reactions of neutral thiols (copolymers P2-1[a-f]), successful conjugations were observed using 0.1 equivalent of the catalyst. However, this amount of catalyst failed to achieve the desired conversions in the reactions of 3-mercaptopropionic acid, mercaptosuccinic acid, cysteamine HCl and L-cysteine HCl. These thiol compounds contain protons that are more labile (lower pKa values) than those of the other thiols. The base catalyst and/or the enolate anion intermediate, a strong base, may preferentially abstract a proton from these highly labile donors, hindering the (re)generation of thiolate anion and quenching the reaction cycle. To circumvent this issue, an excess TEA was added to neutralize these acidic groups. For example, the use of 1.1 equivalents of TEA in the reaction of 3-mercaptopropionic acid yielded >99% conjugation.

As indicated in Table IV-3, the use of slight excess (1.3 equivalents) of the thiol reactant yielded quantitative conjugations to copolymer P2-1. However, the reaction of 2-methyl-2-propanethiol (copolymer P2-1b) resulted only in ~83% conjugation. Increasing the amount of thiol to 5.0 equivalents resulted in >99% alkene conversion as observed in 1H NMR spectroscopy.

The efficiencies of the Michael-type thiol addition reactions were indicated by the complete disappearance of the vinyl resonances (5.7-6.5 ppm) as well as the appearance of new resonances due to the conjugated thiols in the 1H NMR spectra. For example, copolymer P2-1e shows resonances at 3.5 and 4.75 ppm from the methylene and hydroxyl protons, respectively, of the conjugated 2-mercaptoethanol (Figure IV-10). In addition, new resonances between 2.5-2.75 are attributed to the methylene protons located next to the thioether linkage. 1H NMR and FT-IR spectra of all functionalized
copolymers are available in the Appendix B. The corresponding molecular weights and polydispersities are shown in Table IV-3. All functionalized copolymers maintained low polydispersities that are comparable to those of the precursor copolymer P2-1. For example, the SEC traces for copolymers P2-1 and P2-1e are shown in Figure IV-11.

**Figure IV-10.** $^1$H NMR spectrum (in DMSO-d$_6$) of copolymer P2-1e. Quantitative conjugation of the thiol is indicated by the complete disappearance of the vinyl resonances at 5.7-6.5 ppm as well as the appearance of new resonances associated to the conjugated thiol.
Figure IV-11. SEC trace (RI) of hydroxyl-functionalized copolymer 2-1e and its precursor copolymer P2-1.
**Table IV-4.** Copolymers Prepared Via Free Radical-mediated Addition of Thiols to Precursor Copolymer **P2-2** Using ADVN as Free Radical Source and DMSO as Solvent (100 mg mL$^{-1}$ Copolymer Concentration, 20 hrs, 50 °C, Ene : Thiol : Initiator = 1: 10 : 0.3).

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Thiol</th>
<th>Conversion (%)</th>
<th>Mn(theo)</th>
<th>Mn(SEC)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2-2a</td>
<td><img src="image1" alt="diagram" /></td>
<td>&gt;99</td>
<td>41,300</td>
<td>47,900$^a$</td>
<td>1.16</td>
</tr>
<tr>
<td>P2-2b</td>
<td><img src="image2" alt="diagram" /></td>
<td>&gt;99</td>
<td>43,000</td>
<td>50,200$^a$</td>
<td>1.15</td>
</tr>
<tr>
<td>P2-2c</td>
<td><img src="image3" alt="diagram" /></td>
<td>&gt;99</td>
<td>46,900</td>
<td>73,000$^a$</td>
<td>1.14</td>
</tr>
<tr>
<td>P2-2d</td>
<td><img src="image4" alt="diagram" /></td>
<td>0</td>
<td>45,300</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P2-2e</td>
<td><img src="image5" alt="diagram" /></td>
<td>&gt;99</td>
<td>41,600</td>
<td>52,600$^a$</td>
<td>1.13</td>
</tr>
<tr>
<td>P2-2f</td>
<td><img src="image6" alt="diagram" /></td>
<td>98</td>
<td>44,100</td>
<td>41,700$^b$</td>
<td>1.01</td>
</tr>
<tr>
<td>P2-2g</td>
<td><img src="image7" alt="diagram" /></td>
<td>&gt;99</td>
<td>44,800</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P2-2h</td>
<td><img src="image8" alt="diagram" /></td>
<td>83</td>
<td>41,400</td>
<td>42,300$^b$</td>
<td>1.17</td>
</tr>
<tr>
<td>P2-2i</td>
<td><img src="image9" alt="diagram" /></td>
<td>&gt;99</td>
<td>47,000</td>
<td>41,900$^b$</td>
<td>1.03</td>
</tr>
<tr>
<td>P2-2j</td>
<td><img src="image10" alt="diagram" /></td>
<td>0</td>
<td>46,500</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ Determined using SEC (DMF).

$^b$ Determined using aqueous SEC
The functionalization of P2-2 was conducted in solution via thermal initiation following literature procedure. The thiol-ene addition reactions (alkene/thiol/initiator = 1/10/0.3) were performed in DMSO solution. The hydrophobic thiols with the exception of thiophenol were successfully conjugated to the precursor copolymer P2-2 (see Table IV-4). Thiophenol is an aromatic thiol and its thyl radical is more stable than that of an alkyl thiol. In contrast to thiol Michael-type addition, the free radical additions of polar thiols to P2-2 are sensitive to the chemical structure of the thiols. The primary thiols (2-mercaptoethanol, 3-mercaptoproprionic acid and cysteamine HCl) yielded quantitative conversions of the allyl groups. On the other hand, the reaction of the bulkier thiols (1-thioglycerol and mercaptosuccinic acid) resulted in less than 100% conjugation. Lastly, attempts to conjugate L-cysteine HCl to P2-2 using various solvents such as DMSO, DMSO/buffer and DMSO/DI water mixtures failed. It should be noted that the pH of the reaction mixtures was carefully adjusted to the acidic range, and monitored before and after reactions to make certain that the thiol groups were protonated.

Potential Applications

In the previous sections, the efficiency of sequential RAFT polymerization, structopendent isocyanate coupling, and thiol click addition in preparing well-controlled structure was demonstrated. The reaction sequence described here offers yet another synthetic route for preparation of stimuli-responsive amphiphilic block copolymers for utility in controlled/targeted release, diagnostics, formulation, water remediation, enhanced oil recovery, etc. Such additional ways of introducing selected hydrophobic, pH-, salt- or temperature-responsive segments will add to the “toolbox” available to chemists for constructing reversible micelles, vesicles, rods, and other nanostructures.
Some obvious extensions of these “proof of concept” studies are generalized in Scheme IV-3.

**Scheme IV-3.** Conceptual examples demonstrating the utility of the synthetic pathway involving sequential isocyanate and thiol-ene reactions from a RAFT-synthesized polymeric scaffold.

A simple demonstration of how changes in amphiphilicity of the precursor (scaffold) block copolymer affect hydrodynamic dimensions in water is depicted in Figure IV-12. The RAFT precursor copolymer **P2** is water soluble but is transformed into amphiphilic, micelle-forming block copolymers **P2-1** and **P2-2** with hydrodynamic diameters of 30 nm and 38 nm by reactions with 2-(acryloyloxy)ethylisocyanate and allylisocyanate, respectively (Scheme III-5). The thiol click addition of mercaptosuccinic acid yields the responsive block copolymer **P2-1h** with pH-dependent transition from unimers of approximately 3 nm to micelles of 50 nm.
Figure IV-13 shows comparison of the pH-dependent behavior of carboxylic acid (P2-1g), amine (P2-1i) and zwitterionic (P2-1j) conjugates of P2-1 prepared by thiol click additions of 3-mercaptopropionic acid, cysteamine and L-cysteine, respectively.

**Figure IV-12.** Size distributions as measured by DLS (in aqueous solutions) of (a) precursor copolymer P2 (9.0 nm), (b) acrylate-functionalized copolymer P2-1 (29.7 nm), (c) allyl-functionalized copolymer P2-2 (37.8 nm), and (d) mercaptosuccinic acid-functionalized, pH-responsive copolymer P2-1h (2.9 nm at pH 7, 49.8 nm at pH 3) (1.0 mg mL$^{-1}$ copolymer concentration).
Figure IV-13. pH response of functional copolymers P2-1g (carboxylic acid), P2-1i (amine) and P2-1j (zwitterion) as measured by DLS in water at 1.0 mg mL$^{-1}$ copolymer concentrations.

Copolymer P2-2i was prepared from the thiol click addition of cysteamine to the allyl-substituted precursor P2-2. The formation of interpolyelectrolyte complexes of this protonated cationic block copolymer with transfer Ribonucleic Acid (tRNA) was studied (see Appendix B). Gel electrophoresis experiments indicate behavior dependent on cationic block length (N/P ratio) and suggest application as alternative scaffolds to those recently reported for gene delivery of RNA or DNA.$^{45, 222, 309, 310}$

In a final example, glycopolymer derivatives were prepared by conjugating 1-thio-$\beta$-d-glucose via the free radical pathway. Synthetic glycopolymers are promising materials for designing therapeutic carriers since sugar-binding proteins such as lectins found on cell surfaces are responsible for various intercellular recognition processes.$^{311-314}$ Synthetic polymeric glycoconjugates exhibit enhanced signal recognition by lectins due to the multivalency of the saccharide moieties, a behavior termed as cluster glycoside
Data from lectin-binding assay of the glycopolymers are shown in the Appendix B.

Section III. Reversible “Self-Locked” Micelles from a Zwitterion-Containing Triblock Copolymer

Overview

Amphiphilic, zwitterionic copolymers are a class of macromolecules which have unique behavioral characteristics arising from interaction of the constituent charged segments with ionic species in the surrounding environment. Conformational changes in response to external stimuli including ionic strength and pH have been studied extensively for the two major classes of polyzwitterions, namely polyampholytes and polybetaines. Interpolyelectrolyte complex formation and the “anti-polyelectrolyte” effect in aqueous media are two specific characteristics which can be capitalized on for construction of technologically advanced materials. The ability to synthesize precise polyzwitterionic block, star, and graft copolymers with potential for assembly into organized structures in water has until recently been limited. However, the advent of CRP techniques and more RAFT polymerization which can be conducted in a facile manner directly in water now allows the level of control of molecular weight, segmental sequence, polydispersity and monomer selection necessary for regulated assembly.

Although relatively few studies utilizing RAFT-synthesized zwitterionic block copolymers have appeared in the literature, unique biocompatible and stimuli-responsive characteristics in aqueous media suggest untapped potential for such materials in biologically relevant applications. Technologically promising cross-linking methodologies, some of which are reversible, have also been developed within the past
few years that allow “locking” of assembled (multimeric) nanostructures for delivery of diagnostic and therapeutic agents. In many cases disassembly can be triggered at specific sites in response to physiological conditions, leading to both controlled release of active agents and subsequent biological elimination of the constituent unimers.

As part of the continuing efforts to develop effective cross-linking chemistries in aqueous media, reversible “self-locked” (cross-linked) micelles assembled from a pH-responsive, zwitterionic triblock copolymer are described here. The copolymer was prepared via aqueous RAFT polymerization utilizing a trithiocarbonate chain transfer agent and monomers with zwitterionic, anionic, and neutral functionality which can be polymerized directly in water without the need of protecting groups.43,295

**RAFT Polymerization**

A well-defined triblock copolymer composed of a permanently hydrophilic poly(N,N-dimethylacrylamide) (PDMA) block, a salt-responsive poly(3[2-(N-methyl acrylamido)ethyl(dimethylammonio)propanesulfonate] (PMAEDAPS) middle block and a pH-responsive poly(3-acrylamido-3-methyl butanoic acid) (PAMBA) block was prepared as illustrated in Scheme IV-5 by aRAFT polymerization. EMP was chosen as the chain transfer agent since it works quite well with acrylamido monomers, affording excellent control over molecular weight and polydispersity, resisting hydrolytic degradation, and allowing rapid monomer conversion at low temperatures.43,295 The PDMA\(_{102}\)-b-PMAEDAPS\(_{64}\)-b-PAMBA\(_{69}\) triblock was prepared by first synthesizing the PDMA macroCTA and sequentially blocking with MAEDAPS and AMBA. The chain extension polymerizations were carried out in 0.5 M NaCl aqueous solution in order to prevent precipitation of the block copolymers. Molecular weights and PDIs of the PDMA macroCTA (Mn=10,300 g mol\(^{-1}\), PDI=1.05), the intermediate diblock (Mn=28,200 g mol\(^{-1}\)
PDI=1.04) and the final triblock (Mn=40,000 g mol$^{-1}$, PDI=1.03) were obtained from MALLS-SEC analysis. Details of the synthesis, assembly and characterization are described in the experimental section.

**Scheme IV-4.** Aqueous RAFT polymerization PDMA-\textit{b}-PMAEDAPS-\textit{b}-PAMBA triblock copolymer.

*Formation and Characterization of “Self-Locked” Micelles*

Scheme IV-5 illustrates the aqueous solution behavior of this responsive system. Above pH 4.6 and in the presence of 0.5 M salt, the triblock copolymer is molecularly dissolved and exists in unimeric form. Lowering solution pH to a value below 4.6 leads to the formation of multimeric micelles. Under these conditions, the AMBA block that forms the micelle core is protonated and hydrophobic while the DMA and MAEDAPS blocks remain soluble and are in the corona of the micelle. The added salt disrupts the electrostatic interaction of the zwitterions allowing the MAEDAPS middle block to have an extended, more hydrated conformation. Removal of the salt by dialysis allows the zwitterionic moieties of the polybetaine segments to interact, resulting in “self-locking” of the structure. (Here, the term “self-locking” is used to clearly distinguish this process from crosslinking methods that require introducing an extrinsic crosslinking agent in
order to maintain nanostructural integrity.) This crosslinking can be readily reversed by introduction of electrolyte at physiological pH.

**Scheme IV-5.** Reversible self-assembly of “self-locked” micelles of PDMA-\(b\)-PMAEDAPS-\(b\)-PAMBA triblock copolymer.

Previously the McCormick research group and others have reported shell crosslinking of charged block copolymer nanostructures by formation of interpolyelectrolyte complexes (IPECs),\(^{67, 322, 323, 325}\) for example by adding a positively charged polyelectrolyte to a negatively charged corona of an assembled polymeric micelle or vesicle. Unlike their classical, covalently crosslinked counterparts, both IPEC complexes and the “self-locking” polybetaine-based systems reported here can be disassembled to their unimeric states by simply increasing ionic strength. However, an advantage of using betaine-containing triblock copolymer is that the hydrophilic, sterically-stabilized corona present throughout the self cross-linking process appears to preclude undesirable inter-particle aggregation often observed with the interpolymer complexes.\(^{322, 323, 325}\)
Figure IV-14. Hydrodynamic diameter ($D_h$) distribution of triblock copolymer P3 (0.1 wt %): (a) unimers (0.5 M NaCl at pH 7), (b) non-crosslinked micelles (0.5 M NaCl at pH 4), (c) “self-locked” micelles at pH 4 (no salt), (d) “self-locked” micelles at pH 6 (no salt); and (e) after addition of salt to “self-locked” micelles at pH 6-7.

The formation of “self-locked” (shell crosslinked) micelles was accomplished as follows: the triblock copolymer (0.1 wt %) was first dissolved in 0.5 M NaCl solution at pH 7 and sequentially dialyzed against: (i) 0.5 M NaCl solution at pH 7 (1 day) to ensure complete dissolution, (ii) 0.5 M NaCl solution at pH 4 (1 day) to protonate the AMBA block and promote self-assembly, (iii) decreasing NaCl concentrations in pH 4 solutions (3 days) and (iv) DI water with pH adjusted to 4 (3 days) to finally obtain the “self-locked” micelles. Figure IV-14 shows the average hydrodynamic diameters ($D_h$) as determined by DLS after each stage of the above process. At pH 7, the triblock copolymers exist as unimers (a) having hydrodynamic diameters of approximately 10 nm while the micelles have unimodal, nearly identical sizes of approximately 35 nm before (b) and after (d) completion of crosslinking. (The same sizes and size distributions from the assembly of 0.1 and 1.0 wt% solutions of the triblock copolymer were also observed.
Temperature changes over the range 20 to 60 °C have no effect on the micelle dimensions.) Upon adjusting the pH of the solution containing the “self-locked” micelles to 6 (deprotonating the AMBA units), the assembly temporarily remains intact, but slowly dissociates into unimers in 2-3 days. By contrast, addition of salt at solution pH ranging from 5-7 results in immediate disassembly to the unimers (e) shown by the dashed line in Figure IV-14.

![Figure IV-15](image)

**Figure IV-15.** The variation of apparent hydrodynamic diameter with pH of the PDMA-\(b\)-PMAEDAPS-\(b\)-PAMBA triblock copolymer (Cp = 0.1 wt %).

The importance of the balance of segments in the triblock polymer is also demonstrated during the self-locking process as shown in Figure IV-14. Micelles formed at pH 4 in 0.5 M NaCl have nearly identical dimensions to the “self-locked” micelles following salt removal by dialysis as previously mentioned. Adjustment of pH from 4 to 6 in the latter stages of dialysis, which might be expected to yield relatively larger dimensions due to ionization of the AMBA block, serves to decrease \(D_h\), if only by a few nm. Changes in the core volume might be insignificant due to relatively short AMBA block as balanced by the nonionic and betaine blocks. An alternative explanation based
on strongly interacting anionic/zwitterionic blocks in the absence of salt, previously postulated for styrene-based sulfonate/sulfobetaine copolymers, was suggested by a reviewer.\textsuperscript{319}

![Figure IV-16](image-url)

**Figure IV-16.** Hydrodynamic size distribution of PDMA-\textit{b}-PMAEDAPS diblock copolymer (a) before and (b) after dialysis against DI water to remove salt (C\textsubscript{p} = 0.1 wt %).

It is important to note that the presence of the neutral, hydrophilic DMA sequence and its composition relative to the nearly equal number of acidic and betaine blocks are key to the stimuli-reversible assembly observed. First of all, the 102:64:69 experimentally determined ratio of DMA:MAEDAPS:AMBA units in the respective blocks confers water solubility at pH 7 to the unimers in 0.5 M NaCl. The pH dependence on aggregation behavior of the triblock copolymer in water was followed by DLS (see Figure IV-15). Unimers with D\textsubscript{h} of \textasciitilde10 nm are observed at neutral pH and maintain that size as pH is progressively lowered; at pH 4.6 (close to the pKa of the AMBA block\textsuperscript{326}) a sharp transition occurs; assembled structures ranging in size from 30-35 nm form as pH is
further lowered. The relatively hydrophobic MAEDAPS units at progressively lower ionic strength likely contribute to some reorganization of the micelle core initially formed by protonated AMBA segments.

It is also instructive that the precursor PDMA$_{102}$-b-PMAEDAPS$_{64}$ diblock copolymer in aqueous saline solutions will form aggregates upon dialysis, behavior anticipated from poorly hydrated zwitterionic blocks upon removal of salt. These aggregates, however, have bimodal size distributions (for example see Figure IV-16) which are not consistent in size or composition. This behavior contrasts the facile formation of the uniform self-assembled micellar structure from the triblock which we attribute to the sufficiently hydrated DMA block which prevents intermicellar zwitterionic interactions.

The ratio of hydrophilic to hydrophobic components of a copolymer determines the shape of self-assembled nanostructures.$^{327}$ The apparent radius of gyration, $R_G$, of the micelles is 12.5 nm as shown in the Zimm plot (Figure IV-17). Using the same polymer solutions, the corresponding average apparent hydrodynamic radius, $R_H$, obtained from DLS is 15.0 nm. The ratio $R_G/R_H$ (0.83) is indicative of spherical or micellar structure which is the expected shape based on the relative block lengths of the triblock copolymer.$^{221, 328-330}$ From the Zimm plot and the molecular weight of the unimers, the aggregation number for the self-assembled micelles was calculated to be 29. The micellar structure is also confirmed by transmission electron microscopy (TEM) (Figure IV-18). The particle size measured by TEM (100-200 nm), however, is significantly larger than the value obtained from light scattering experiments. The discrepancy is attributed to inter-micellar aggregation during solvent evaporation.
Figure IV-17. Zimm plot for the self-assembled PDMA-b-PMAEDAPS-b-PAMBA triblock copolymer micelles (Cp=0.2-1.0 wt %, pH=4).

Figure IV-18. TEM image of shell cross-linked PDMA-b-PMAEDAPS-b-PAMBA triblock copolymer micelles (0.1 wt %).
The self-assembly of the triblock copolymer was followed using $^1$H NMR spectroscopy (Figure IV-19). For the diblock copolymer precursor, there are no discernable differences in the spectra of the unimers (with the added salt) and aggregates (without salt). The signals attributed to protons in the MAEDAPS block decrease in intensity but are still prominent in the solution containing the aggregates. The zwitterionic block that is responsible for aggregation of the diblock copolymer is thus sufficiently solvated to not restrict motion on the NMR time scale. This observation is in accordance with a previously reported zwitterionic copolymer system.\textsuperscript{316} In the case of the triblock copolymer, however, when micelles are formed, the signals due to the AMBA block are significantly attenuated and the associated resonances broaden and shift upfield. As with the diblock, the signals due to the MAEDAPS block of the shell cross-linked micelles are also visible.

The formation of hydrophobic domains during micelle formation was also investigated by fluorescence spectroscopy using pyrene and 8-anilino-1-naphthalene sulfonic acid (ANS). Pyrene is a hydrophobic probe that shows a red shift in its excitation spectra and a change in the relative intensities of its emission bands, while ANS exhibits a blue shift accompanied by an increase in its emission intensity when confined within a more hydrophobic environment.\textsuperscript{330-334} The pyrene excitation band shifts from 330 nm to 337 nm in the presence of triblock copolymer at pH 7 (Figure IV-20). However, the excitation spectrum is only slightly red shifted when the micelles are formed at pH 4. The ratio of $I_3$ and $I_1$ in the pyrene emission spectrum (Figure IV-21(a)) increases from 0.62 to 0.77 when solution pH was adjusted from 7 to 4. This increase in the intensity ratio signifies a change in polarity of the immediate surroundings of the probe which can be correlated to micelle formation.\textsuperscript{334} Zwitterionic aggregates do not favor solubilization of
the highly hydrophobic probe,\textsuperscript{316} and hence, it can be postulated that the pyrene preferentially goes into the core of the triblock copolymer micelle which is formed by the protonated AMBA block. When ANS is added to a solution containing pre-assembled triblock copolymer micelles at pH 4, the fluorescence spectrum is similar to that at pH 7 where the copolymers exist as unimers. In this case, the probe is likely situated on the zwitterionic shell of the micelles. The interaction of the charged groups of ANS and the copolymer prevent the fluorescent probe from diffusing into the core of the micelles. To circumvent this problem, the unimers and the fluorescent probe were first mixed and the pH of the solution was slowly adjusted to form the micelles. With the added polymer, the emission wavelength of maximum intensity shifts from 522 nm to 505 nm (Figure IV-21[b]). When the micelles are formed, a further blue shift to 478 nm is observed along with a large increase in fluorescence intensity.
Figure IV-19. $^1$H NMR spectra of unimers and self-assembled aggregates of diblock and triblock copolymers.
**Figure IV-20.** Pyrene excitation fluorescence spectra with the triblock copolymer (Cp = 1.0 wt %, [Pyrene] = 0.54 μM).
Figure IV-21. (a) Pyrene ($\lambda_{ex} = 339$ nm) and (b) ANS ($\lambda_{ex} = 360$ nm) emission fluorescence spectra with the triblock copolymer ($C_p = 1.0$ wt %, [pyrene] = 0.54 µM, [ANS] = 50 µM).
CHAPTER V

CONCLUSIONS

Section I. Direct RAFT Polymerization of an Unprotected Isocyanate-Containing Monomer and Subsequent Structopendant Functionalization Using Click-Type Reactions

Successful direct RAFT homopolymerization of 2-(acryloyloxy)ethyl isocyanate (AOI) has been accomplished. The polymerization conditions were optimized utilizing TBP as the chain transfer agent by varying stoichiometry of monomer/CTA and varying the polymerization temperature and the solvent. Direct RAFT polymerization of AOI requires a neutral CTA and relatively low reaction temperature to yield AOI homopolymers with good polydispersities. Efficient side-chain functionalization of AOI homopolymers can be achieved through reactions with amines, thiols and alcohols resulting in urea, thiourethane and urethane linkages, respectively. Reaction with amines and thiols (in the presence of base) are fast, quantitative and efficient. However, the reaction with alcohols utilizing dibutyltin dilaurate (DBTDL) catalyst is relatively slow but can proceed to completion. Selective reaction pathways for the side-chain derivatization of PAOI homopolymers using difunctional ethanolamine and mercaptoethanol were identified. Work is now underway in our laboratories to extend these model studies of structopendant isocyanate “click”-type reactions of RAFT-based polymers to thiol, hydroxyl and amine terminated synthetic and biological (macro)molecules.

Section II. Structopendent Transformations of RAFT Block Copolymers via Sequential Isocyanate Reaction and Thiol Michael Addition

The combination of RAFT polymerization and sequential reactions involving carbamate formation and thiol-ene click addition to modify the pendent groups of well-
defined copolymer proves to be a facile, modular approach for synthesis of a library of functional copolymers from a single copolymer scaffold. The RAFT technique provides extensive options for monomer selection while the isocyanate-hydroxyl group reactions and thiol additions to alkene offer highly versatile routes for structopendent group transformations. The model thiols of this study reacted efficiently via Michael-type and free radical-mediated thiol-ene addition reactions. The modular capability of the method allows the attachment of various groups to the polymer chains and hence preparation of multifunctional scaffolds. This approach may be envisioned for the conjugation of thiol-containing molecules such as proteins and other bio-relevant species in combination with other moieties for targeting, imaging and therapeutics. Efforts to prepare multifunctional polymeric architectures using the strategy outlined here are being explored in our laboratories.

Section III. Reversible “Self-Locked” Micelles from a Zwitterion-Containing Triblock Copolymer

In this work, we have demonstrated a facile cross-linking method for forming polymeric micelles from a well-defined ABC triblock polymer synthesized directly in water that does not require the addition of an external cross-linking agent. The formation of “self-locked” micelles is induced by first lowering solution pH below the pKa of the AMBA block at a salt concentration sufficient to hydrate the MAEDAPS block and subsequently removing the salt by dialysis. The reversible crosslinks from the interaction of the zwitterionic groups are readily broken by the addition of electrolyte, resulting in a micelle disassembly into unimers. This triblock and other related systems have potential as nanocarriers for controlled delivery of therapeutic and diagnostic agents.
APPENDIX A
MODELS REACTIONS OF ISOCYANATE WITH AMINES, THIOLS AND ALCOHOLS

Efficiency and selectivity of isocyanate reactions were investigated using cyclohexylisocyanate (CHI) and small molecule amines, thiols and alcohols as model compounds. Stoichiometric amounts of amine, thiol or alcohol reactants were added dropwise to CHI solutions in CDCl$_3$ (~5 wt%, 0.37 M). The reactions with thiols were catalyzed by triethylamine (TEA, 0.5 wt%, 50 mM) whereas dibutyltin dilaurate (DBTDL, 0.1 wt%, 1.6 mM) catalyzed the reactions with alcohols. The solutions were stirred overnight at room temperature. The efficiency and selectivity of the reactions were probed by examining the carbonyl signals in both FT-IR and $^{13}$C NMR spectra. Characteristic signals for urea, urethane and thiourethane were first identified using monofunctional amine, alcohol and thiol, respectively (Figures A1 and A4). An identical procedure was followed for reactions with ethanolamine and mercaptoethanol (Figures A2, A5-A6).

The reaction of isocyanates with amines does not require the addition of a catalyst and is selective over those of alcohols. Similarly, the base-catalyzed reaction of isocyanates and thiols is highly efficient and occurs in preference to hydroxyl reactions. Urethane formation can be catalyzed by DBTDL and proceeds at a relatively slower rate as compared to reactions of amines or thiols. When both the hydroxyl and the thiol groups are present in the reaction mixture, the latter has been shown to deactivate the DBTDL catalyst which affects urethane formation.$^{118}$ There are conflicting reports in literature regarding the role of the tin(IV) catalyst in urethane formation.$^{118}$ Free thiols are relatively unreactive towards isocyanates under neutral conditions and even in the
presence of DBTDL catalyst. This is not surprising as free thiols are less nucleophilic compared to hydroxyl groups. Once deprotonated, however, the thiolate anions become excellent nucleophiles and react immediately with isocyanates.

Unlike the reactions of more reactive aryl isocyanates, the reactions of CHI and mercaptoethanol yielded both urethane and thiourethane linkages. Thiourethane was preferentially formed in the absence of catalyst (see Figures A3 and A7), however the reaction of hydroxyl groups was favored with increasing amounts of DBTDL catalyst.

The $^{13}$C NMR spectrum of the product from the reaction of CHI and mercaptoethanol (NCO:SH:OH=2:1:1) with 0.5 wt% TEA and 0.1 wt % DBTDL is shown in Figure A8 for comparison. The peak positions are slightly shifted compared to the mono-capped adducts. Note that this spectrum was taken in a mixture of CDCl$_3$, CD$_3$COCD$_3$ and CD$_3$OD as solvent since the product was not completely soluble in CDCl$_3$ alone.
Scheme A1. Model reactions of isocyanate with amines, thiols and alcohols.

1. \( \text{NCO} + \text{H}_2\text{N}^+\text{R} \rightarrow \text{NH}^+\text{N}_\text{R} \)

2. \( \text{NCO} + \text{HO}^-\text{R} \rightarrow \text{O} \)

3. \( \text{NCO} + \text{HS}^-\text{R} \rightarrow \text{S} \)

4. \( \text{NCO} + \text{HO}^-\text{R} \text{NH}_2 \rightarrow \text{OH} \)

5. \( \text{NCO} + \text{HO}^-\text{R} \text{SH} \rightarrow \text{OH} \)

6. \( \text{NCO} + \text{HO}^-\text{R} \text{SH} \rightarrow \text{OH} \)

7. \( \text{NCO} + \text{HO}^-\text{R} \text{SH} \rightarrow \text{SH} \)

8. \( 2 \times \text{NCO} + \text{HO}^-\text{R} \text{SH} \rightarrow \text{SH} \)
Figure A1. FT-IR spectra showing the carbonyl region of the product from the reaction of cyclohexylisocyanate (CHI) with 3-propylamine, ethanol (with 0.1 wt% DBTDL) and ethanethiol (with 0.5 wt% TEA).
Figure A2. FT-IR spectra showing the carbonyl region of the product from the reaction of cyclohexylisocyanate (CHI) with ethanolamine and mercaptoethanol with DBTDL (0.1 wt%) and TEA (0.5 wt%).
Figure A3. FT-IR spectra showing the carbonyl region of the product from the reaction of cyclohexylisocyanate (CHI) and mercaptoethanol with selected amounts of added DBTDL as catalyst. Direction of arrow indicates increasing catalyst concentration.
Figure A4. $^{13}$C NMR spectra (in CDCl$_3$) of the product from the reaction of (A) cyclohexylisocyanate and 3-propylamine, (B) cyclohexylisocyanate and ethanol (with 0.1 wt% DBTDL as catalyst) and (C) cyclohexylisocyanate and ethanethiol (with 0.5 wt% TEA as catalyst).
Figure A5. $^{13}$C NMR spectrum (in CDCl$_3$) of the product from the reaction of cyclohexylisocyanate and ethanolamine.

Figure A6. $^{13}$C NMR spectrum (in CDCl$_3$) of the product from the reaction of cyclohexylisocyanate and mercaptoethanol with 0.5 wt% TEA as catalyst.
Figure A7. $^{13}$C NMR spectra (in CDCl$_3$) of the product from the reaction of cyclohexylisocyanate and mercaptoethanol with selected amounts of added DBTDL catalyst: (A) no catalyst (spectrum taken after 3 days), (B) 0.004 wt% (after 24 hrs), (C) 0.065 wt% (after 24 hours) and (D) 0.19 wt% (24 hrs). (continued in next page)
13C NMR spectra (in CDCl₃) of the product from the reaction of cyclohexylisocyanate and mercaptoethanol with selected amounts of added DBTDL catalyst: (A) no catalyst (spectrum taken after 3 days), (B) 0.004 wt% (after 24 hrs), (C) 0.065 wt% (after 24 hours) and (D) 0.19 wt% (24 hrs).

**Figure A7.** (*continued from previous page*)
Figure A8. $^{13}$C NMR spectrum (in CDCl$_3$/CD$_3$COCD$_3$/CD$_3$OD) of the product from the reaction of cyclohexylisocyanate and mercaptoethanol with TEA (0.5 wt%) and DBTDL (0.1 wt%) (NCO:SH:OH=2:1:1). The reaction was conducted in CDCl$_3$. The product precipitated out. After completion of the reaction, the precipitate was dissolved in CDCl$_3$/CD$_3$COCD$_3$/CD$_3$OD for NMR analysis.
APPENDIX B

NMR AND FT-IR SPECTRA OF COPOLYMERS FROM SEQUENTIAL FUNCTIONALIZATION RAFT BLOCK COPOLYMERS

Figure B1. $^1$H NMR spectrum (in D$_2$O) of PDMA-CEV macroCTA. Degree of polymerization (DP) was calculated using peak areas of methyne proton (f) of the DMA backbone and the terminal methyl group (a, inset) of the RAFT CTA.

Figure B2. $^1$H NMR spectrum (in D$_2$O) of PDMA-b-PHEA (P2) diblock copolymer.
Figure B3. $^1$H NMR spectrum of copolymer P2-1a in DMSO-$d_6$.

Figure B4. $^1$H NMR spectrum of copolymer P2-1b in DMSO-$d_6$. 
Figure B5. $^1$H NMR spectrum of copolymer P2-1c in DMSO-$d_6$.

Figure B6. $^1$H NMR spectrum of copolymer P2-1d in DMSO-$d_6$. 
Figure B7. $^1$H NMR spectrum of copolymer P2-1f in DMSO-$d_6$.

Figure B8. $^1$H NMR spectrum of copolymer P2-1g in DMSO-$d_6$. 
Figure B9. $^1$H NMR spectrum of copolymer P2-1h in DMSO-$d_6$.

Figure B10. $^1$H NMR spectrum of copolymer P2-1i in D$_2$O.
**Figure B11.** $^1$H NMR spectrum of copolymer P2-1j in D$_2$O.

**Figure B12.** $^1$H NMR spectrum of copolymer P2-2a in DMSO-$d_6$. 
Figure B13. $^1$H NMR spectrum of copolymer P2-2b in DMSO-$d_6$.

Figure B14. $^1$H NMR spectrum of copolymer P2-2c in DMSO-$d_6$. 
Figure B15. $^1$H NMR spectrum of copolymer P2-2e in DMSO-$d_6$. 

Figure B16. $^1$H NMR spectrum of copolymer P2-2f in DMSO-$d_6$. 
Figure B17. $^1$H NMR spectrum of copolymer P2-2g in D$_2$O (at pH 7).

Figure B18. $^1$H NMR spectrum of copolymer P2-2h in D$_2$O (at pH 7).
Figure B19. $^1$H NMR spectrum of copolymer P2-2i in D$_2$O (at pH 5).
Figure B20. FT-IR absorption spectra (NaCl plate) of (a) PDMA$_n$-b-PHEA$_m$ (P2) diblock copolymer precursor, (b) acrylate-functionalized PDMA$_n$-b-PHEA(acrylate)$_m$ (P2-1) copolymer and (c) allyl-functionalized PDMA$_n$-b-PHEA(ene)$_m$ (P2-2) copolymer. Characteristic bands associated to the reactions of hydroxyl groups with the isocyanate-containing alkenes are identified.
Figure B21. FT-IR absorption spectra (NaCl) of functionalized copolymers P2-1(a-j).
Figure B22. FT-IR absorption spectra (NaCl) of functionalized copolymers P2-2(a-c) and P2-2(e-i). Conjugations of thiophenol (P2-2d) and L-cysteine (P2-2j) failed.
Complexation of Copolymer P2-2i with tRNA

Transfer RNA (tRNA) solution (1 µL, 20 µM) was pipetted into seven 200 µL centrifuge tubes. This was diluted with the appropriate amount of nuclease free water and phosphate buffer solution (2 µL, 82.5 mM, pH 7.4). Aliquots of copolymer solution were added into each tube for the corresponding N/P ratios. The final volume was 8.25 µL giving 20 mM phosphate buffer and approximately 2.5 µM tRNA concentrations. All samples were vortexed immediately and allowed to incubate for 30 minutes at room temperature. Agarose gel (1%) was prepared and pre run for 30 minutes prior to well loading. The running buffer was 1 X trisborate-EDTA, 8 M Urea. Each sample was diluted with 8.25 µL of 2 X trisborate-EDTA, 8 M Urea solution (no dye). The gel was allowed to run for 30 minutes (93 Volts) and was visualized through ethidium bromide staining (see Figure B23).

![Figure B23](image.png)

**Figure B23.** Agarose gel image of tRNA interpolyelectrolyte complexes (IPECs) with the amine-functionalized copolymer P2-2i at various nitrogen to phosphate (N/P) ratios.
**Lectin-Binding Assay of Glycopolymer**

The glycopolymer was prepared via free radical addition of sodium 1-thio-β-D-glucose to an allyl-containing precursor copolymer (PDMA$_{112}$-b-PHEA(allyl)$_{23}$, Mn=15,200 PDI=1.21). The copolymer (200 mg, 0.30 mmol ene), thiol (650 mg, 3.0 mmol) and AIPD (32 mg, 0.09 mmol) were dissolved in dioxane/water mixture. The pH of the solution was adjusted to 4-5 with 0.1 M HCl. After purging with N$_2$ for 1 hr at 0 °C, the mixture was heated at 40 °C for 24 hrs. The copolymer was purified by dialysis against acidic water for 3 days followed by lyophilization (Mn=17,800 PDI=1.25, conversion >99%).

To a solution of FITC-Con A in phosphate buffer (3 mL, 24 nM, pH 7.4) was added copolymer solution (2 µL, 8 mM). After mixing, the solution was equilibrated at room temperature for 15 minutes. The fluorescence emission intensity at 517 nm of the solution was then measured using 490 nm as the excitation wavelength. Additional aliquot of copolymer was added every 15 minutes and the incremental decrease in fluorescence intensity was monitored.

FITC-Con A has an intrinsic emission peak at 517 nm which is quenched upon binding of the glycopolymer. The relative change in fluorescence intensity of FITC-Con A as a function of glucose concentration was plotted (Figure B24a). The lectin-binding affinity or association constant ($K_a$) of the glucose-functionalized copolymer was estimated using Scatchard plot as described by the following equation:

$$\frac{[\text{sugar}]F_0}{\Delta F} = \frac{[\text{sugar}]F_0}{\Delta F_{\text{max}}} + \frac{F_0}{\Delta F_{\text{max}}K_a}$$

where [sugar] is the glucose concentration, $F_0$ is the initial fluorescence intensity and $\Delta F$ is the change in fluorescence intensity. The obtained $K_a$ value ($7.5 \times 10^4$ M$^{-1}$) is comparable to those of other synthetic glycopolymers reported in the literature.
Figure B24. (a) Variation of fluorescence intensity from the binding of glycopolymer with fluorescently-labeled lectin (FITC-Con A) and (b) the resulting Scatchard plot for the estimation of the association constant, $K_a \left(7.5 \times 10^4 \text{ M}^{-1}\right)$. 

![Diagram](image-url)
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