Self-Assembly and Gold Nanoparticle Cross-Linking of Stimuliresponsive Block Copolymers Synthesized by Reversible Addition-Fragmentation Chain Transfer Polymerization

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SELF-ASSEMBLY AND GOLD NANOPARTICLE CROSS-LINKING OF STIMULI-RESPONSIVE BLOCK COPOLYMERS SYNTHESIZED BY REVERSIBLE ADDITION-FRAGMENATATION CHAIN TRANSFER POLYMERIZATION

by

Adam Eugene Smith

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

May 2010
ABSTRACT

SELF-ASSEMBLY AND GOLD NANOPARTICLE CROSS-LINKING OF STIMULI-RESPONSIVE BLOCK COPOLYMERS SYNTHESIZED BY REVERSIBLE ADDITION-FRAGMENTATION CHAIN TRANSFER POLYMERIZATION

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The ability of amphiphilic block copolymers to self-assemble into various morphologies in aqueous solution in response to specific stimuli has attracted widespread interest for potential applications as targeted drug delivery and diagnostic vehicles. Stimuli-responsive block copolymers afford a facile method for tuning the hydrophilic mass fraction to provide access to various solution morphologies. Reversible addition-fragmentation chain transfer (RAFT) polymerization provides the ability to prepare stimuli-responsive block copolymers while maintaining precise control over the macromolecular characteristics (molecular weight, copolymer composition, functionality, etc.) that dictate nanostructure morphology.

This work may be divided into four sections. In the first section the synthesis and thermally-responsive self-assembly behavior of poly[2-(dimethylamino)ethyl methacrylate\textsubscript{73}-block-(N-isopropylacrylamide)\textsubscript{99}] (P(DMAEMA\textsubscript{73}-b-NIPAM\textsubscript{99})) is discussed. At elevated temperatures, P(DMAEMA\textsubscript{73}-b-NIPAM\textsubscript{99}) exhibited a reversible vesicle formation in aqueous solution. Simply mixing a pH 7.4 vesicle solution at 50 °C with a solution of NaAuCl\textsubscript{4} led to the formation gold nanoparticle (AuNP)-“decorated” vesicles.
The second study details the preparation of a series of DMAEMA and NIPAM block copolymers. Controlling block lengths, solution pH, and NaCl concentration to elicit changes in the hydrophilic mass fraction resulted in specific morphological changes upon thermally-induced assembly. At 68 wt% DMAEMA, P(DMAEMA$_{165}$-b-NIPAM$_{102}$) self-assembled into simple core-shell micelles (58 nm). Increasing the DMAEMA content to 48 wt% lead to a mixture of spherical micelles (78 nm) and worm-like micelles (D=50-100 nm, L=400-500 nm). Further increasing to 36 wt% DMAEMA produced vesicular structures (179 nm). The associated nanostructures were subsequently shell cross-linked above the critical aggregation temperature via the in situ formation of AuNPs to yield assemblies with long term aqueous stability.

In the third section the reversible gold nanoparticle cross-linking of polymeric vesicles derived from a RAFT-generated, thermally-responsive diblock copolymer, P(DMAEMA$_{165}$-b-NIPAM$_{435}$), is reported. Vesicles were first self-assembled above the critical aggregation temperature of the diblock copolymer and subsequently cross-linked by the in situ AuNP formation in the tertiary amino-functionalized vesicle shell. The cross-linking was then reversed by the addition of the thiols, cysteamine or a thiolated poly(ethylene glycol) (PEG-SH), capable of inducing a ligand exchange on the surface of the AuNP to free the bound polymer chains. The sizes of the thiol-stabilized AuNPs produced during the ligand exchange with both cysteamine and PEG-SH were found to be ~ 8 nm.

In the fourth study, dually-responsive block copolymers of (N,N-diethylaminoethyl methacrylate and NIPAM capable of “schizophrenic” aggregation in aqueous solution were synthesized via aqueous RAFT polymerization. The nanoassembly
morphologies, dictated by the hydrophilic mass fraction, were systematically controlled by the polymer block lengths, solution pH, and temperature. Both P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) (52.5 wt% NIPAM) and P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>) (70.8 wt% NIPAM) self-assembled into PDEAEMA-core, PNIPAM-shell spherical micelles (~42 and 52 nm, respectively) at temperatures below the lower critical solution temperature (LCST) of PNIPAM and at solution pH values greater than the pK<sub>a</sub> of PDEAEMA. The two block copolymers, however, display quite different temperature-responsive behavior at pH < 7.5. At elevated temperatures (> 42 °C) P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) formed spherical micelles (~52 nm) with hydrophobic PNIPAM cores stabilized by a hydrophilic PDEAEMA shell. By contrast, P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>) assembled into vesicles (~200 nm) above 38 °C.
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CHAPTER I
INTRODUCTION

Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization

The discovery of living polymerization techniques, the first of which was described by Szwarc in 1956, represents a significant breakthrough in the ability to prepare advanced polymer architectures. Unlike conventional chain polymerizations, living polymerizations proceed in the absence of termination and chain transfer reactions. The suppression of termination and chain transfer allows for the synthesis of well-defined polymers of predetermined molecular weight, narrow molecular weight distributions, and advanced architectures. Examples of the advanced architectures accessible using controlled polymerization techniques are shown in Figure I-1 and include statistical (1), alternating (2), AB diblock (3), ABA (4) and ABC (5) triblock, tapered block (6), graft (7), and star (8) structures. Although the necessary control of molecular weight, molecular weight distributions, and polymer architecture could be achieved with anionic, cationic, or group transfer polymerization methods, such techniques are applicable to limited monomer choices and require stringent reaction conditions, most notably the absence of water. The desire to prepare advanced architectures obtainable in living polymerizations, while maintaining the robust reaction conditions and diverse monomer selection of conventional free radical polymerizations, led to the rapid development of controlled/living free radical polymerization (CRP) techniques.
Figure I-1. Advance (co)polymer architectures accessible via CRP techniques.
In order to prepare well-defined polymers by a free radical process, it is necessary to reduce termination reactions. Because free radicals terminate at nearly diffusion controlled rates, this goal can only be accomplished by employing very low radical concentrations. Similar to the early ionic systems, the CRP techniques establish an equilibrium strongly favoring dormant chains over propagating chains in an effort to minimize the radical concentration. The lower radical concentration leads to a reduction in the overall rate of polymerization; however, the rate of termination is suppressed to a greater extent due to a second order dependence on radical concentration. An optimized CRP has less than 10 % dead chains as opposed to a conventional free radical polymerization in which over 99 % of the chains are terminated by coupling and/or disproportionation.2

The major controlled radical polymerizations can be divided into two types based on the mechanism by which they activate/deactivate chains (Scheme I-1). The first type of controlled radical polymerization relies on a reversible termination mechanism to impart control. Various techniques based on reversible chain termination have been developed including iniferters3,4, stable free radical polymerization (SFRP)5,6 (Scheme I-1, I), and atom transfer radical polymerization (ATRP)7,8 (Scheme I-1, II). The second type of CRP relies on a degenerate chain transfer process and is best exemplified by reversible addition-fragmentation chain transfer (RAFT) (Scheme I-1, III).
Since the initial literature report by the CSIRO group in 1998, the RAFT polymerization method has proven to be perhaps the most versatile of the CLRP techniques, allowing the polymerization of virtually all classes of vinyl monomers under a wide range of reaction conditions, including polymerization in homogenous aqueous solution. The versatility of RAFT polymerization has resulted in rapidly increasing utilization as demonstrated by the increasing number of publications over the last 11 years (Figure I-2), including reviews on the RAFT process, aqueous RAFT, the mechanism of RAFT polymerization, RAFT in heterogeneous media, and computational studies.
Figure I-2. Number of scientific publications on RAFT/MADIX (search performed on 10-30-2009 utilizing Scifinder with the following keywords: reversible addition fragmentation chain transfer and/or MADIX and/or RAFT polymerization).

The Mechanism of RAFT Polymerization

Unlike SFRP and ATRP which are based on the reversible deactivation of propagating radical chains, RAFT relies on a series of reversible chain transfer reactions to impart control. The accepted RAFT mechanism is shown in Scheme I-2. Since RAFT is essentially conventional radical polymerization conducted in the presence of a chain transfer agent (CTA), initiation can be accomplished with traditional initiators such as azo compounds, peroxides, redox initiating systems, photoinitiators, and γ-radiation. Figure I-3 lists some common initiators utilized in RAFT polymerization. The primary radical, I•, is generally believed to add to monomer prior to addition to the CTA due to the high relative concentration of monomer to CTA. This assumption, however, may prove incorrect in cases with highly reactive CTAs or lower monomer concentrations. For most RAFT polymerizations, the concentration of initiator relative to CTA is kept low to ensure a majority of the chains are initiated by CTA fragments (R•) as initiator-
derived chains have a negative effect on the control of the molecular weight of the resulting polymer. Additionally, due to the exponential decomposition of conventional thermal initiators, primary radicals are continuously produced throughout the polymerization possibly leading to bimolecular termination. The continuous production of radicals also has the beneficial effect of replenishing any radicals lost to termination events and aids in maintaining reasonable polymerization rates.

\[\begin{align*} &\text{AIBN} \\ &\text{V-70} \\ &\text{V-501} \\ &\text{VA-044} \end{align*}\]

**Figure I-3.** Common thermal initiators employed in RAFT polymerizations.

After reaction of the primary radical \(I^*\) with monomer to give a propagating oligomeric chain (10), the CTA (11) reacts with \(P_n^*\) to give an intermediate radical (12). This intermediate radical can fragment to yield the CTA and \(P_n^*\), or, if the correct CTA is chosen, fragmentation to form a polymeric macroCTA (13) and a new radical species, \(R^*\) (14), is favored. The pre-equilibrium is defined as the time required for all \(R^*\) fragments to add monomer units to form propagating chains, \(P_m^*\), and is governed by the four rate constants \(k_{add}, k_{add}, k_\beta\) and \(k_\beta\). In order to achieve narrow molecular weight distributions, the pre-equilibrium must be completed early in the reaction for all the chains to enter the main equilibrium at the same time. This is analogous to other living
polymerization systems in which initiation is assumed to occur quantitatively and instantaneously.

\[
\begin{align*}
\text{I.} & \quad \text{Initiator} \quad \mathbf{k_d} \quad \xrightarrow{} \text{2I}^* \\
& \quad \text{I}^* + \text{Monomer} \quad \xrightarrow{} \text{P}_n^* \\
\text{II.} & \quad \text{P}_n^* + \text{Z-C-S-P}_n \quad \frac{k_{\text{add}}}{k_{\text{add}}} \quad \xrightarrow{} \text{P}_m - \text{S-C-S-R} \quad \frac{k_{\beta}}{k_{\beta}} \quad \xrightarrow{} \text{Z-C-S-P}_n + \text{R}^* \\
& \quad 10 \quad 11 \quad 12 \quad 13 \quad 14 \\
\text{III.} & \quad \text{R}^* + \text{Monomer} \quad \xrightarrow{} \text{P}_m^* \\
& \quad \text{P}_n^* + \text{Monomer} \quad \xrightarrow{} \text{P}_{n+x}^* \\
\text{IV.} & \quad \text{P}_m^* \quad \text{Monomer} \quad \frac{k_{\text{add}}}{k_{\text{add}}} \quad \xrightarrow{} \text{P}_m - \text{S-C-S-P}_n \quad \frac{k_{\beta}}{k_{\beta}} \quad \xrightarrow{} \text{Z-C-S-P}_n + \text{P}_m^* \\
& \quad 15 \quad 16 \quad 17 \\
\text{V.} & \quad \text{I}; \text{R}; \text{P}_n^*; \text{P}_m^* \quad \mathbf{k_t} \quad \xrightarrow{} \text{Dead Polymer} \\
\end{align*}
\]

**Scheme I-2.** The accepted RAFT mechanism.

Once the pre-equilibrium is complete, the polymerization enters the main equilibrium. This stage involves the degenerative transfer of the thiocarbonylthio end group between propagating chains through the formation and fragmentation of an intermediate radical (16). The exchange between active and dormant chains is established by the rapid fragmentation of the intermediate radical in both directions allowing for the controlled, intermittent addition of monomer to each chain with equal probability. Most monomer consumption occurs during the main equilibrium and the number of monomer additions can vary depending on reaction conditions. It has been suggested, however,
that for most RAFT polymerizations, less than one monomer is added to the propagating chains per transfer step.\textsuperscript{26}

As in all “living” polymerization techniques, RAFT works to limit the number of irreversible termination events by minimizing the instantaneous concentration of active species available for termination. As in all free radical processes, however, termination events occur through radical coupling and disproportionation and can be directly related to the starting initiator concentration. When the primary mode of termination is bimolecular combination, the number of dead chains is equal to half the number of initiator derived chains. In the case where disproportionation is the dominant mode of termination, the number of dead chains is equal to the total number of initiator derived chains.\textsuperscript{9} Termination of the intermediate radicals through radical coupling and disproportionation has also been shown, but the experimental conditions were not typical for RAFT polymerizations.\textsuperscript{27-29} The RAFT process effectively limits the number of termination events and the high $[\text{CTA}]_0/[\text{I}]_0$ commonly used prevents the number of dead chains from exceeding 5\%.\textsuperscript{10}

\textit{The RAFT Chain Transfer Agent}

The key component in controlled RAFT polymerization is the CTA.\textsuperscript{30, 31} The CTAs used are thiocarbonylthio compounds and have the general structure RSC(=S)Z. Examples of RAFT agents span all thiocarbonylthio families including dithioesters, xanthates, dithiocarbamates, and trithiocarbonates. Figure I-4 shows generic structures of CTA classes while Figure I-5 illustrates specific examples of CTAs that have been employed in the synthesis of stimuli-responsive polymers. For each monomer to be polymerized by RAFT, an appropriate choice of CTA must be made given the exact
balance that must be struck between the reversible addition and fragmentation reactions outlined in the previous section. Improper CTA selection can cause a loss of control, significant retardation, a prolonged induction period, and/or complete inhibition of polymerization. RAFT agents are chosen based on the nature of the Z and R groups, so it is important to understand what effect each has on the polymerization of a specific monomer.

The main role of the Z group is to activate the thiocarbonyl double bond for radical addition in order to prevent extensive propagation from occurring before the initial chain transfer event.\textsuperscript{30} Inherently, the Z group also aids in stabilization, and hence lifetime, of the intermediate radicals formed in the pre- and main equilibria of the RAFT process. Increased activation of the thiocarbonyl double bond increases the likelihood of propagating chains will add to the CTA, allowing fewer monomers to add to the growing polymer chains between transfer events. Over-stabilization of the intermediate radicals, however, can lead to slow fragmentation resulting in retardation of the polymerization\textsuperscript{32} and a higher probability of intermediate radical termination\textsuperscript{33, 34}

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

**Figure I-4.** Generic structures of RAFT chain transfer agents.
Figure I-5. Examples of RAFT CTAs utilized in the synthesis of stimuli-responsive (co)polymers.

Although the Z group contributes to the reactivity of the thiocarbonyl throughout a RAFT polymerization, the contribution from the R group is encompassed completely in the pre-equilibrium. The role of the R group is to effectively fragment from the pre-equilibrium intermediate radical and subsequently reinitiate polymerization. The stability of the expelled R• (14) must be greater than or equal to the oligomeric radical P_n• (10) to allow for fragmentation from the intermediate radical; however, the reactivity of R• must be high enough to rapidly reinitiate polymerization of monomer. As an example of this interplay between the roles of the R group, Donovan et al. observed a significant induction period for the cumyl dithiobenzoate (CDB) (CTA4)-mediated polymerization of N,N-dimethylacrylamide (DMA). Because the cumyl radical is
expected to be a much better leaving group than the acrylamido chain end, the induction period was attributed to slow reinitiation.

**Molecular Weight Control by RAFT Polymerization**

Several conditions must be met in order for a RAFT polymerization to control molecular weight. The two most important criteria are a sufficiently high ratio of CTA to initiator and proper CTA selection for the monomer of choice. According to the RAFT mechanism, there are two potential sources from which polymer chains are derived, initiator fragments (9) and the CTA leaving group (14). As such, the theoretical number-averaged molecular weight ($M_n$) can be defined as

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

where $[M]_0$ is the initial monomer concentration, $M_{MW}$ is the molecular weight of the monomer, $\rho$ is the monomer conversion, $[CTA]_0$ is the initial CTA concentration, $f$ is the initiator efficiency, $[I]_0$ is the starting initiator concentration, $k_d$ is the initiator decomposition rate constant, and $CTA_{MW}$ is the molecular weight of the CTA.\(^{10,11}\) In a well-designed RAFT polymerization with a high CTA to initiator ratio, the fraction of initiator-derived chains will be less than 5 % and the term for such chains can be neglected.\(^{10}\) This allows simplification of Equation 1 to Equation 2.

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

From this relationship, molecular weight increases linearly with conversion allowing for the synthesis of tailored polymers with predetermined molecular weights and low polydispersities (PDIs).
**Synthesis of Block Copolymers via RAFT**

RAFT is a versatile method that easily facilitates the synthesis of functional block copolymers due to the retention of the thiocarbonylthio group on the chain end. AB diblock copolymers are prepared by the addition of a second monomer to a macroCTA. Sequential monomer addition for block copolymer formation is not generally used in RAFT because it has been shown that chain end functionality decreases with increasing reaction times.\(^{36}\) Polymerizations are stopped before quantitative monomer conversion is reached, and the resulting polymer is then isolated, purified, and utilized as a macroCTA in the polymerization of the subsequent monomer as shown in Scheme I-3. Other block structures such as ABC and ABA may also be prepared using the same strategy.

**Scheme I-3.** Synthesis of AB diblock copolymers via RAFT.

In order for a blocking reaction to be efficient, the propagating radical of the first block must fragment efficiently and add to the second monomer thus making the proper order of monomer addition imperative.\(^{37, 38}\) In addition to the preparation of ABA triblock copolymers through three sequential monomer addition steps, analogous materials may be prepared utilizing difunctional RAFT agents as shown in Figure I-6.
Triblock copolymers prepared from difunctional RAFT agents can be prepared in two synthetic steps and usually have higher blocking efficiencies than from monofunctional CTAs requiring three synthetic steps. Well-defined block copolymers may also be prepared by functionalizing pre-polymers produced by an alternative polymerization method with thiocarbonylthio groups. For example, this strategy was used by Li and coworkers in the McCormick Research group who prepared poly(ethylene oxide-block-dimethylacrylamide-block-N-isopropylacrylamide) from dithiobenzoate functionalized poly(ethylene oxide).³⁹

![Chemical structure of RAFT agents](image)

**Figure I-6.** Difunctional RAFT agents facilitating ABA triblock copolymer formation in two synthetic steps.

*Considerations for RAFT Polymerizations in Aqueous Media*

While the economic and environmental advantages are obvious, successful RAFT polymerization directly in aqueous media can only be achieved by elimination of competitive reactions during polymerization. First and foremost is the hydrolysis of the thiocarbonylthio moiety of RAFT CTAs. Since CTAs are simply sulfur analogues of esters, it is not surprising that they are susceptible to hydrolysis. Levesque et al. examined the hydrolytic stability of several thiocarbonylthio compounds in mild conditions (20-35 °C, pH 7.5-8.5).⁴⁰ Both the pH and temperature affected the rate of hydrolysis of the compounds, with increased hydrolysis observed with increasing
temperature and pH. Thomas et al. conducted a detailed study regarding the effect of solution pH on the hydrolysis of small molecule CTAs and macroCTAs.\textsuperscript{41} Since water is in large excess, the hydrolysis of the CTA functionality can be assumed to be zero-order with respect to water. The rate of CTA hydrolysis can, therefore, be expressed in terms of the apparent rate constant, $k_{\text{hyd}}$, and the CTA concentration as shown in Equation 3.

\begin{equation}
-\frac{d[\text{CTA}]}{dt} = k_{\text{hyd}} [\text{CTA}]
\end{equation}

(3)

Pseudo first-order rate plots of the hydrolysis of 4-cyanopentanoic acid dithiobenzoate (CTP) (\textbf{CTA1}) and two sodium 2-acrylamido-2-methylpropanesulfonate (AMPS) macroCTAs made with CTP gave reasonably good fits to Equation 3.\textsuperscript{41} The rate of hydrolysis of all three species increases at higher pH, consistent with the finding of Levesque et al.\textsuperscript{40} Additionally the rate of hydrolysis of the two AMPS macroCTAs was dramatically reduced as compared to the small molecule, CTP. This behavior was attributed to steric hindrance of the dithioester to the attack of water molecules, analogous to the well-known steric effects observed for carboxylic ester hydrolysis.

It is important to note that the thiocarbonylthio compounds are not tolerant of all functionality. The reaction of a thiocarbonylthio compound with primary and secondary amines is known to be first order with respect to the concentration of thiocarbonylthio and display a second order dependence on the amine concentration.\textsuperscript{40, 42} Thomas et al. also investigated the effect of aminolysis on CTA stability by conducting aminolysis experiments using CTP in buffered media with ammonium hydroxide to give an ammonia concentration of 5 mM.\textsuperscript{41} The fraction of CTP remaining was determined as a function of time at pH 5.5 and 7.0. As noted in their report, the loss of CTP was due to both aminolysis as well as hydrolysis with the rate equation given as
\[- \frac{d[C\text{TA}]}{dt} = k_{\text{hyd}}[\text{CTA}] + k_a[\text{CTA}][\text{NH}_3]^2 \]  

(4)

where \( k_a \) is the aminolysis rate constant and \([\text{NH}_3] \) is the concentration of ammonia in solution. Taking the time dependence of CTA hydrolysis and aminolysis and the faster hydrolysis of the small molecule CTA into account, Eq. 5 was developed to determine the theoretical molecular weight under conditions where both hydrolysis and aminolysis are active.

\[
[M]_n(t) = \frac{M_{\text{MW}}([M]_0 - [M]_0 e^{-k_p(t-t_{\text{ind}})})}{[\text{CTA}]_{\text{ind}} e^{-(k_{\text{hyd,macro}}+k_{a,\text{macro}}[\text{NH}_3]^2)(t-t_{\text{ind}})}}
\]  

(5)

Considering these complications, monomers containing primary or secondary amines are often thought to be precluded from direct polymerization by RAFT. It has been shown, however, that only unprotonated amines will react with the thiocarbonylthio functionality and aminolysis can be greatly reduced by lowering pH.\(^{40,42}\) Recently, our group reported the polymerization of a primary amine containing monomer, \(N-(3\text{-aminopropyl})\text{methacrylamide (APMA)}\), and the subsequent chain extension with \(N\text{-isopropylacrylamide (NIPAM) (M1)}\).\(^{43}\) The polymerizations were mediated by CTP in a water/dioxane mixture with a pH between 4 and 5 to minimize the hydrolysis and aminolysis of the CTA moiety. Subsequently Xu et al. in our lab, polymerized APMA directly in water (pH 4-5) using a mPEO-macroCTA (trithiocarbonate functionality).\(^{44}\) The mPEO-PAPMA was then chain extended with 2-(diisopropylamino)ethyl methacrylate (DPAEMA) (M20) to form a pH-responsive triblock copolymer. Armes and coworkers have recently reported the RAFT polymerizations of 2-aminoethyl methacrylate (AMA), another primary amine-containing monomer in dimethyl sulfoxide (DMSO) using CDB.\(^{45}\)
In addition to the susceptibility of the thiocarbonylthio moiety to hydrolysis, critical monomer classes can also prove problematic under certain polymerization conditions. Specifically, (meth)acrylamido monomers are capable of undergoing hydrolysis to produce primary or secondary amines which can react with the CTA as mentioned above. Given the high monomer concentration relative to CTA, even a few percent of monomer hydrolysis can result in complete loss of the thiocarbonylthio end group, and hence loss of control of the polymerization. Thomas et al. examined the effect of hydrolysis of acrylamide (AM) on the loss of CTA. In order to minimize hydrolysis of AM (release of ammonia), Thomas and coworkers found it imperative to conduct the polymerization under acidic conditions to maintain control.

**Stimuli-Responsive Block Copolymers Synthesized by RAFT**

Since the advent of polymer science as a discipline, chemists have sought to design and synthesize “smart” macromolecules that respond to external signals such as temperature, pH, electrolytes, light, and mechanical stress. Such stimuli-responsive polymers have found a plethora of applications in widely diverse fields including, but not limited to: biomedicine, optics, electronics, diagnostics, and in formulation of pharmaceuticals and cosmetics. In many cases, synthetic polymers have been constructed to mimic the behavior of an enormously diverse array of biological polymers including proteins, nucleic acids, polysaccharides, and their naturally occurring conjugates.

Prior to the development of controlled radical polymerization (CRP) techniques, functional monomer selection, broad polydispersity, and lack of structural and molecular weight control limited synthesis of systems with requisite primary, secondary,
and tertiary features for conformational response and assembly featured in stimuli-responsive biomolecules. In terms of application to all stimuli-responsive systems, and in particular those of biological relevance, RAFT is currently the most versatile of the CRP techniques. The powerful synthetic tools developed for RAFT polymerization and subsequent transformations now allow polymerization of highly functional monomers under benign conditions (often in water at ambient temperature without the need of protecting groups) to afford complex, but highly controlled architectures with tailored ranges of response to external stimuli.

**Monomers for Thermally-Responsive Blocks**

Temperature-responsive (co)polymers exhibit a volume phase transition at a critical temperature, which causes a sudden change in the solvation state. Such (co)polymers, which become insoluble upon heating, have a lower critical solution temperature (LCST). Conversely, systems which 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 the entropic effects of the dissolution due to the ordered state of water molecules in the vicinity of the polymer and the enthalpic effects due to hydrogen bonding and hydrophobic interactions. These transitions are observed as coil-to-globule transitions.
Figure I-7. Monomers used for synthesizing thermally-responsive (co)polymers.

*N-Isopropylacrylamide* NIPAM (M1) is among the most widely studied neutral monomers in all of polymer science due in most part to the readily accessible LCST of \( \sim 32^\circ C \) of PNIPAM in water, just below physiological temperature (37 \(^\circ C\)). The LCST of PNIPAM can be tuned by controlling the molecular weight or via incorporation of hydrophilic or hydrophobic groups.\(^{47, 48}\) While a description of every report of the polymerization of NIPAM by RAFT would take volumes, important milestones are detailed here which impact potential application as drug delivery vehicles.

Ganachaud et al. reported the RAFT polymerization of NIPAM in 2000.\(^{49}\) Low PDI (1.1 < PDI <1.5) PNIPAM was synthesized at 60 \(^\circ C\) with 2,2'-azobisisobutyronitrile
(AIBN) (I1) as the radical initiator and with benzyl dithiobenzoate (BDB) (CTA3) and CDB (CTA4) in benzene and 1,4-dioxane, respectively. Schilli et al. subsequently utilized benzyl and cumyl dithiocarbamates for the homopolymerization of NIPAM in 1,4-dioxane as 60 °C. The same group also reported one of the first block copolymers comprised of PNIPAM, chain extending a poly(acrylic acid) macroCTA with NIPAM in methanol using AIBN as the radical source.

Convertine et al. in our labs first demonstrated the room temperature RAFT polymerization of NIPAM in dimethyl formamide (DMF) using 2-dodecylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid (DMP) (CTA12) as the CTA and an azo initiator, 2,2'-azobis(4-methoxy-2,4-dimethyl valeronitrile) (V-70) (I2), capable of initiation at 25 °C. Prior to this, only a few examples of room temperature RAFT polymerizations had been reported. The Mn vs. conversion plot showed the characteristic linear evolution of Mn with conversion and PDIs remaining low throughout the polymerization.

Yusa et al. subsequently reported the RAFT polymerization of NIPAM in a methanol/water mixture (8/2 v/v) using a NaAMPS macroCTA and 4,4’-azobis(4-cyanopentanoic acid) (V-501) (I3) at 70 °C. Kinetics of the block polymerization in an 8/2 v/v methanol-d4/D2O at 70 °C were monitored by 1H NMR spectroscopy. The pseudo first-order kinetic and conversion vs. time plots were linear over the first 100 minutes of the polymerization, consistent with a “living” polymerization mechanism. For polymerization times greater than 100 minutes, downward curvature was attributed to a decreasing concentration of active radicals.
Building on our previous work\textsuperscript{52} and that of Yusa et al.\textsuperscript{56}, our group reported the first polymerization of NIPAM in water. Convertine et al. used the difunctional thiolester carbonate $2\text{-}(1\text{-carboxy-1-methyl-ethylsulfanylthiocarbonylsulfanyl})\text{-}2\text{-methylpropionic acid (CMP)}$ (\textbf{CTA6}) and a novel monofunctional, water-soluble CTA, $2\text{-ethylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid (EMP)}$ (\textbf{CTA7}), directly in water using the azo initiator $2,2\text{'}\text{-azobis[2-(2-imidazolin-2-yl)propane]}$ dihydrochloride \,(VA-044) \,(14).\textsuperscript{57} A comparatively low CTA to initiator ratio (3:1) was employed due to the relatively long half life of VA-044 at 25 °C. Following a short induction period, the pseudo first-order kinetic plots for the two polymerizations show linear kinetics even at high monomer conversions. In the same report, block copolymers were also synthesized via ambient temperature aqueous RAFT polymerization of NIPAM using mono- and difunctional N,N-dimethylacrylamide (DMA) macroCTAs to produce di- and triblock copolymers, respectively.

Mueller and coworkers subsequently reported the polymerization of NIPAM and AA at ambient temperature using $\gamma$-radiation in the presence of CMP and $3\text{-benzylsulfanylthiocarbonylsulfanyl propionic acid (BPA)}$ (\textbf{CTA10}) in aqueous solution.\textsuperscript{58} Detailed studies of the polymerization of NIPAM mediated by these two thiolester carbonates revealed that both polymerizations proceeded in a controlled fashion.

RAFT polymerization has widely been used to synthesize PNIPAM of controlled molecular weight, low polydispersity, and prescribed $\alpha$- and $\omega$-end group functionality. RAFT-generated PNIPAM has applications in many areas of polymer science including conjugation to biomolecules\textsuperscript{59-65}, stabilization of metal nanoparticles\textsuperscript{66-79}, surface-functionalization of various substrates\textsuperscript{80-87}, and synthesis of star\textsuperscript{88-93}, comb\textsuperscript{94-96}, and
branched \(^{97-101}\) polymers. NIPAM segments also serve as building blocks for forming stimuli-responsive micelles and vesicles.

*Other N-alkyl substituted acrylamides.* While innumerable manuscripts have reported the RAFT polymerization of NIPAM, relatively little work has been done with other thermoresponsive N-alkyl substituted acrylamides. A report of Cao and coworkers is one of the most exhaustive studies of the RAFT polymerization of N-alkyl substituted acrylamides.\(^{102}\) The monomers studied included the hydrophilic monomer DMA and the temperature-responsive monomers NIPAM, N-n-propylacrylamide (nPAM) (\(M_2\)), N,N-diethylacrylamide (DEA) (\(M_3\)), and N-ethylmethylacrylamide (EMA) (\(M_4\)). The \(M_n\) vs. conversion plots for the polymerization of nPAM and DEA show a linear dependence with negative deviations from the theoretically predicted molecular weights. These deviations were attributed to initiator-derived chains. This study suggested that the disubstituted acrylamido monomers were better controlled than the monosubstituted counterparts under the same polymerization conditions, a fact that was attributed to the higher reactivity and formation of more stable intermediate radicals due to the stronger electron-donating effects.

Block copolymers of the N-alkyl substituted acrylamides were also studied. In most cases, chain extension of the disubstituted PDMA, PDEA, and PEMA resulted in a mixture of block copolymers and unreacted homopolymer whereas extension of the monosubstituted PNIPAM and PnPAM resulted in mostly successful blocking experiments. These results further indicate that reactivity differs for nearly all N-alkylacrylamide monomers due to changes in the number and structure of the substituents. Using what was learned from the blocking experiments, Cao et al.
synthesized a tetrablock copolymer P(nPA_{129}-b-NIPAM_{52}-b-EMA_{63}-b-DMA_{184}) and studied the thermal-responsiveness in water using turbidity measurements. Both multiblock copolymers had M_n values close to theoretical values and low PDIs (< 1.25). Zhu and coworkers recently followed this initial work with a more detailed study of the solution properties of an ABC triblock copolymer comprised of copolymer P(nPA_{124}-b-NIPAM_{80}-b-EMA_{44}) which also showed a three-step temperature transition.\(^{103}\)

Mori and coworkers first reported the BDB-mediated RAFT polymerization of the thermoresponsive amino acid derivative N-acryloyl-L-proline methyl ester (A-Pro-OMe) (M8) in chlorobenzene at 60 °C using AIBN as the initiator.\(^{104}\) The M_n of the P(A-Pro-OMe) increased linearly with monomer conversion and was in agreement with theoretical values with PDIs between 1.13 and 1.22. P(A-Pro-OMe) exhibited an LCST of 15.0 °C in water (1 mg/mL). In an effort to modulate the LCST, A-Pro-Ome was also copolymerized with DMA under the same conditions to increase the hydrophilicity of the copolymer. Subsequently, Mori et al. performed studies detailing the effect of reaction conditions (solvent, \([\text{CTA}]_0/\text{[I]}_0\)) on the polymerization of A-Pro-OMe as well as \(^1\text{H}\) NMR and MALDI-TOF experiments to show the retention of active chain ends necessary for chain extension.\(^{105}\) Block copolymers were next synthesized by the chain extension of PDMA and PS macroCTAs with A-Pro-OMe and the chiroptical and thermosensitive properties of P(DMA-b-A-Pro-OMe) were compared to P(A-Pro-OMe) and a random copolymer of A-Pro-Ome and DMA. The CD spectra showed that the ability of the block copolymer to form ordered structures was greater than that of either the random copolymer or the homopolymer. Recently, Mori and coworkers synthesized polymers of N-acryloyl-L-proline and N-acryloyl-4-trans-hydroxy-L-proline, performed post-
polymerization modification to give the corresponding methyl esters, and studied their phase transition in water.\textsuperscript{106}

Another example of a disubstituted acrylamide that displays an LCST in water is N-acryloyl pyrrolidine (NAPy) (M\textsubscript{5}). To date NAPy has been studied solely by Laschewsky and coworkers.\textsuperscript{107, 108} NAPy was successfully polymerized using CDB in toluene at 70 °C to yield PNAPy of 15,000 at 78 % conversion. Additionally poly(t-butyl acrylate) was chain extended with NAPy and the temperature-responsive aqueous solution behavior was studied. Unfortunately, no experimental evidence was provided to confirm the controlled nature of the homo- and copolymerization.

Poly(N-vinyl pyrrolidone) (PNVP) is a well known water-soluble, biocompatible polymer. Recently, Deng et al. polymerized the thermoresponsive NVP analogue N-(2-methacryloyloxyethyl) pyrrolidone (NMP) (M\textsubscript{6}) and studied the effect of molecular weight on the cloud point (CP) of aqueous solutions of PNMP.\textsuperscript{109} 2-Cyanoprop-2-yl(4-fluoro)dithiobenzoate was used to mediate the RAFT polymerization of NMP in anhydrous methanol at 30 °C using (2,4,6-trimethylbenzoyl)diphenylphosphine oxide as a visible light degradable radical source. Pseudo first-order kinetic curves exhibited linear dependence of the polymerization on radiation time after a short induction period. The molecular weight of PNMP vs. conversion plot also showed a linear dependence with PDIs between 1.1 and 1.2 for most of the polymerization. Additionally, chain extension of the PNMP macroCTA demonstrated retention of the active dithioester chain ends as evidenced by near quantitative block formation. Subsequent light scattering experiments demonstrated a significant dependence of the temperature-response on PNMP molecular weight with the CP decreasing from 71.5 °C at 20.6 kDa to 52.8 °C at 105.4 kDa.
Another cyclic disubstituted acrylamide for synthesizing stimuli-responsive (co)polymers is N-acryloylpiperidine (NAPi) (M7). Hubbell and coworkers polymerized NAPi in the presence of 2-[(2-phenyl-1-thio)thio]propanoic acid in 1,4-dioxane at 90 °C for 24 hours using AIBN. The observed $M_n$ was in agreement with theoretical values and an SEC trace of the homopolymer showed a symmetric, unimodal peak; however, pseudo first-order and $M_n$ vs. conversion plots were not reported. Subsequent block formation using a N-acryloylmorpholine again showed agreement with theoretical molecular weight calculations and PDIs were below 1.3.

Monomers for pH-Responsive Block Copolymers

Polymers containing ionizable groups along or pendant to their backbone are often termed “polyelectrolytes”. There are two types of pH-responsive polyelectrolytes, weak polyacids and weak polybases. A representative acidic pendant group is the carboxylic group. As the solution pH changes, the degree of ionization of the polymer causes a change in the hydration state of the pendant groups, often leading to aggregation. Weak polyacids such as PAA accept protons at low pH and release protons at neutral and high pH. On the other hand, polybases like poly(4-vinylpyridine) (P4VP) are protonated at high pH and positively ionized at neutral and low pH.
Acrylic, methacrylic, and α-substituted acrylic acids. Acrylic acid (AA) (**M10**) has been polymerized using more CTAs than any other monomer studied to date. The RAFT polymerization of AA can be traced back to the original report by the CSIRO group. In this report, the authors polymerized AA in the presence of 1-phenylethyl dithiobenzoate at 60 °C to achieve a polymer with $M_n = 13,800$ and PDI = 1.23 after 4 h. Following this work, Chong et al. demonstrated the ability to synthesize a block copolymer of AA and n-butyl acrylate (BA) and maintain control of the polymerization.

Claverie and coworkers performed a detailed study on the polymerization of AA using 15 different CTAs from the dithioester, xanthate, trithiocarbonate, and dithiocarbamate families. All polymerizations were carried out in ethanol at 90 °C with V-501 (**I3**) used as the primary radical source. The best overall control was found
for the polymerization conducted in the presence of the phenoxyxanthate and trithiocarbonate derivatives. Subsequently, Loiseau et al. investigated the polymerization in the presence of two trithiocarbonates, dibenzyl trithiocarbonate and (1-phenylethyl)trithiocarbonate in methanol, ethanol, 2-propanol, and 1,4-dioxane.\textsuperscript{113} In general, polymerizations were controlled at low conversion but had negative deviations from the theoretical molecular weight values due to transfer to solvent, especially 2-propanol. Lai and coworkers further demonstrated the ability of trithiocarbonates to successfully polymerize AA in a controlled fashion.\textsuperscript{114} The authors synthesized two novel carboxy-functional trithiocarbonates, CMP and DMP, and polymerized AA in both water and DMF to yield polymers with low PDIs at near quantitative monomer conversion.

In contrast to the numerous reports on the RAFT polymerization of AA, little work has been performed on methacrylic acid (MAA) (M\textsubscript{11}), ethylacrylic acid (EAA) (M\textsubscript{12}), and propylacrylic acid (PrAA) (M\textsubscript{12}). Chong et al. published the first report of the RAFT polymerization of MAA. MAA was polymerized in the presence of poly(methyl methacrylate) (PMMA) and poly(benzyl methacrylate) macroCTAs (M\textsubscript{n} of 3,200 and 1800, respectively).\textsuperscript{111} The block copolymers had low PDIs (< 1.2) but no conversion data was given and since the block copolymer molecular weights were not substantially larger than the macroCTAs (4,700 for P(MMA-\text{-}b\text{-}MAA), and 2,400 for P(BzMA-\text{-}b\text{-}MAA)) the PMAA blocks were oligomeric in nature.

Recently, Yang and Cheng reported the homopolymerizations of MAA and NIPAM as well as their block copolymer synthesis.\textsuperscript{115} Polymerization of MAA was conducted in the presence of carboxymethyl dithiobenzoate (CMDB) (CTA\textsubscript{5}) in
methanol using V-501 as the radical source to give a homopolymer of 13,300 Da with a PDI of 1.3 after 10 h. The molecular weight vs. conversion curve exhibited a linear profile but poorly correlated with theoretical calculations due to higher than expected molecular weight at low conversion. This phenomenon was attributed to the slow fragmentation and a low transfer constant of CMDB, resulting in fragmentation back to the PMAA propagating radical instead of the carboxymethyl radical during the pre-equilibrium. Despite the higher than expected molecular weights, di- and tri-block copolymers were synthesized with reasonable PDIs (1.3-1.4).

Recently, Pelet and Putnam synthesized relatively high molecular weight PMAA (up to 113,900 Da) with low PDIs. The authors investigated the effect of the ratios of 
\[ [M]_0 : [CTA]_0 : [I]_0, \] solvent (methanol vs. water/1,4-dioxane), and pH on the control of the polymerization. It was determined that either methanol or a water/1,4-dioxane mixture at low pH (~3) allow for the synthesis of well-defined, monodisperse PMAA at high conversion. Kinetic analysis of these two systems demonstrated linear pseudo first-order dependence of \( \ln([M]_0/[M]) \) on time as is characteristic of RAFT polymerization.

A successful RAFT homopolymerization of EAA has not been reported to our knowledge. EAA has been copolymerized with maleimide in the presence of 2-phenylprop-2-yl dithiobenzoate in dioxane; however, the copolymerization was uncontrolled with experimental molecular weights significantly higher than predicted and with PDIs between 1.93 and 2.96. Copolymerization of the ethyl ester of EAA, ethyl ethylacrylate, and maleimide resulted in reasonable agreement between experimental and theoretical molecular weights and PDIs < 1.4.
As with EAA, the RAFT polymerization of PrAA has only been reported by one group. Stayton and coworkers first reported the copolymerization of PrAA with NIPAM to give a copolymer responsive to both temperature and pH. The DMP-mediated polymerization was performed in methanol at 60 °C using AIBN as the primary radical source. While the polymerizations gave copolymers with narrow PDIs, the experimental molecular weight was consistently higher than that predicted by theory. At a pH value of 5, increasing PrAA content lead to decreases in the copolymer LCST due to the hydrophobic character of the protonated acid functionality. At pH 6.5, this trend was reversed due to the increased hydrophilicity of the PrAA moiety. Recently, Convertine et al. synthesized a diblock copolymer by the chain extension of poly(2-[[dimethylamino)ethyl]methacrylate) (PDMAEMA) (M17) with a random copolymer of DMAEMA, PrAA, and butyl methacrylate for potential application as a delivery vehicle for siRNA.

Other carboxylic acid functional monomers. In early work by our group, sodium 4-vinylbenzoic acid (VBA) (M14) was polymerized using a poly(styrene sulfonate) (PSS) macroCTA to afford a pH-responsive diblock copolymer (Mn = 18,600, PDI = 1.18) capable of self-assembly. At high pH values the sulfonate and carboxylate moieties were ionized and the block copolymer was molecularly dissolved as unimers (~8 nm). At low pH, however, the VBA was protonated and rendered hydrophobic, leading to self-assembly of the block copolymer into spherical micelles of ~19 nm.

Subsequently, Wang and Lowe polymerized VBA to form homo-, statistical co-, and block copolymers with two phosphonium-based styrenic monomers, namely 4-vinylbenzyl(trimethylphosphonium) chloride (TMP) and 4-
vinylbenzyl(triphenylphosphonium) chloride (TPP). The homopolymerization of VBA was mediated by 2-(2-carboxyethylsulfanylthiocarbonysulfanyl) propionic acid (CPA) (CTA11) in DMSO at 80 °C with AIBN as the radical initiator. Linear pseudo first-order kinetics were observed for two [CTA]₀/[I]₀ ratios. The statistical and block copolymerization also displayed well-behaved kinetics. Having established polymerization conditions for VBA using CPA, Lowe and coworkers synthesized a doubly responsive block copolymer P(NIPAM-b-VBA) and studied the self-assembly in water.

N-Acryloyl derivatives of amino acids which can be synthesized in a facile manner are also viable targets for polymerization by RAFT. Recently, Lokitz and coworkers formed dually-responsive block copolymer incorporating N-acryloylvaline (AVAL) (M16) as the pH-sensitive monomer. First, AVAL was studied for its viability to be polymerized by RAFT in a controlled manner. EMP was used to mediate the polymerization of AVAL directly in water (pH = 6.5) at 30 and 70 °C using azo initiators with appropriate decomposition rates. As expected, the apparent rate of polymerization at 70 °C is significantly higher than that at 30 °C. This is attributed to a larger number of initiator radicals yielding a faster rate of propagation at 70 °C. It should be noted that an induction period is observed at 30 °C. The targeted dually-responsive block copolymer was synthesized by the chain extension of a PDMA macroCTA with varying ratios of AVAL and NIPAM in order to tune the assembly behavior in aqueous solution.

Another carboxylic acid monomer that has been polymerized by RAFT is sodium 3-acrylamido-3-methylbutanoate (AMBA) (M15). Sumerlin et al. first reported the
homopolymerization of AMBA and its block copolymerization with AMPS using CTP in water at 70 °C.\textsuperscript{124} Linear first-order kinetics were achieved with agreement between experimental and theoretical molecular weights and low PDIs (< 1.3). Block copolymers of AMPS and AMBA were then synthesized and studied for pH-responsive self-assembly in water. A more detailed study of the effect of copolymer composition and architecture was subsequently reported.\textsuperscript{125} Additionally, AMPS and AMBA block copolymers have been used in the formation of layer-by-layer films.\textsuperscript{126}

\textit{Acrylate and methacrylate derivatives.} Among the pH-responsive tertiary amines that have been polymerized by RAFT, DMAEMA (M17) has been the most widely studied. Many of the early reports on the RAFT polymerization of DMAEMA were part of larger studies screening monomers capable of polymerization by RAFT.\textsuperscript{9, 111, 127} Xiong et al. performed a detailed study of the polymerization of DMAEMA directly in water at 70 °C with CTP (CTA1) and V-501 (I3) as the CTA and initiator, respectively.\textsuperscript{128} A [CTA]₀:[I]₀ = 3 was found to be optimal for obtaining PDIs below 1.3. The kinetic curve revealed pseudo first-order kinetics at early polymerization times, but at longer times negative deviation was observed, indicative of loss of a steady-state radical concentration. The molecular weight increased linearly with conversion with slight positive deviations from linearity at high conversions. The PDIs for the polymerization of DMAEMA remained low (> 1.3). The synthesized PDMAEMA was used as a macroCTA and steric stabilizer for the chain extension with MMA in a miniemulsion polymerization. Efforts to chain extend the PDMAEMA macroCTA with styrene under similar conditions were not successful.
A more detailed study of the RAFT polymerization of DMAEMA was conducted by Sahnoun and coworkers. At a constant [M]₀ of 2.0 M, four degrees of polymerization (DPs) (100, 200, 350, and 500) were targeted. A slight induction period of ~10 min is evident in the pseudo first-order kinetic plots of the four polymerizations in the presence of 2-cyanoprop-2-yl dithiobenzoate (CPDB) (CTA₂) as well as for the control polymerization performed in the absence on CTA. Due to its presence in both the control experiment as well as the RAFT polymerizations, the induction period was attributed to the presence of oxygen instead of an indication of an issue arising from the RAFT polymerization. The kinetic curves for the RAFT polymerizations show a negative deviation from linearity at long times, indicative of a loss of steady state radical concentration.

A report of the polymerization of N,N-dimethylaminoethyl acrylate (DMAEA) (M₁₈) was published by Huang and coworkers. In this report, three layer polymer particles were synthesized by the sequential RAFT polymerization of NIPAM (M₁) and DMAEA from a trithiocarbonate-functionalized hyperbranched polyglycerol core. Polymerizations were conducted at 65 and 70 °C in 1,4-dioxane using AIBN as the primary radical source. The DMAEA shell was then cross-linked with 1,8-diiodooctane and the effect of cross-linking on the thermal response of the NIPAM corona. At 60% cross-linking, the LCST of the particles increased from 35 °C to 40 °C after the cross-linking reaction.

Yusa and coworkers studied the pH-induced micellization of poly([3-(methacryloylamino)propyl]trimethylammonium chloride-block-N,N-diethylaminoethyl methacrylate) (P(MAPTAC-b-DEAEMA)). A PMAPTAC₆₂ macroCTA was chain
extended with varying lengths of DEAEMA (M19) to give three block copolymers. Copolymers with DEAEMA block lengths of 28 and 53 units assembled into micelles at pH 10.0 in 0.1 M NaCl solution. The block copolymer P(MAPTAC$_{62}$-b-DEAEMA$_{11}$) did not exhibit any appreciable change in the hydrodynamic diameter at pH 10.0 compared to pH 4.0. Fluorescence studies using N-phenyl-1-naphthylamine (PNA) demonstrated the ability of the DEAEMA blocks to sequester PNA in hydrophobic micro-environments above pH 7.0, even for the copolymer for which micellization was not detected.

Manguian et al. studied the RAFT polymerization of DEAEMA and investigated the use of a PDEAEMA macroCTA to stabilize and mediate the emulsion polymerization of styrene in water. DEAEMA was polymerized in the presence of CTP (CTA1) and V-501 (I3) in bulk with 10 % added ethanol at 60 °C. The pseudo first-order kinetic curve was linear for the RAFT polymerization of DEAEMA after an induction period of ~ 1 h. The linear progression of $M_n$ with conversion, good agreement between experimental and theoretical molecular weights, and the low PDIs demonstrated that the polymerization of DEAEMA with CTP proceeds in a controlled fashion. The presence of the dithioester group on the polymer was subsequently confirmed by $^{13}$C NMR and UV-vis spectrophotometry. The protonated PDEAEMA macroCTA was then used to synthesize a diblock copolymer of DEAEMA and styrene under emulsion polymerization conditions.

Several reports have detailed the synthesis of dually-responsive block copolymers using DEAEMA and NIPAM (M1) as pH-responsive and temperature-responsive units, respectively. Additionally, DEAEMA has been copolymerized with DPAEMA to produce copolymers with tunable pH-responsive micellization. Hu and coworkers
copolymcrized DEAEMA and DPAEMA (M20) using CTP and AIBN as CTA and initiator, respectively.\textsuperscript{136} Pseudo first-order kinetic curves for the polymerization of DEAEMA, DPAEMA, and a 60/40 mixture of DEAEMA and DPAEMA showed linear trends with little, if any induction period. Similarly, the $M_n$ vs. conversion plots were linear and PDIs remain low (< 1.2) throughout the polymerization. The macroCTA was subsequently chain extended with N-(2-hydroxypropyl)methacrylamide (HPMA) to yield amphiphilic block copolymers. Potentiometric titrations showed the pK$_b$ value for the block copolymers varied linearly with the amount of DEAEMA in the copolymer and introduction of 0.15 M NaCl shifts the pK$_b$ to higher values due to charge shielding, influencing the equilibrium between protonated and unprotonated moieties. Other examples utilizing DPAEMA as a “smart” building block have also been reported.\textsuperscript{44, 45}

\textit{Vinylpyridines.} The first two reports of the RAFT polymerization of the vinylpyridine monomers appeared in the literature near simultaneously. Yuan and coworkers detailed the use of dibenzyl trithiocarbonate to produce block copolymers of 4VP (M22) and styrene and investigated the morphology of the aggregates after dissolution in DMF and dialysis into water.\textsuperscript{137} The polymerizations of 4VP were carried out in DMF at 60 and 80 °C and reached high conversion (> 85%) in 2.5 h. At the same time, Convertine et al. reported the polymerization of both 2-vinyl pyridine (2VP) (M21) and 4VP.\textsuperscript{138} The CDB-mediated homopolymerizations were carried out in bulk using AIBN as the primary radical source. The linearity of the pseudo first-order rate plot and the $M_n$ vs. conversion plot demonstrated controlled polymerization. A brief induction period of less than 1 h was observed in the pseudo first-order rate plot of 2VP, consistent with previous reports using CDB (CTA4) to mediate RAFT polymerizations.\textsuperscript{34, 35, 124, 139,
To further demonstrate the control of the homopolymerizations of 2VP and 4VP, block copolymers were synthesized by chain extension of P2VP with 4VP and P4VP with 2VP. Subsequent reports of the RAFT polymerizations of 2VP and 4VP have detailed the use of the polymers as a building block for dually-responsive systems when copolymerized with NIPAM\textsuperscript{66, 141, 142}, as a stabilizing agent for metal nanoparticles\textsuperscript{66, 143}, and in the preparation of nanocomposites of montmorillonite.\textsuperscript{144}

\textit{N,N-Dimethylbenzylvinylamine.} Mitsukami et al. reported the first polymerization of N,N-dimethylbenzylvinylamine (DMBVA) (M\textsuperscript{23}) by RAFT polymerization.\textsuperscript{120} Block copolymers of DMBVA and (ar-vinylbenzyl)trimethylammonium chloride (VBTAC) were prepared using CTP (CTA\textsubscript{1}) and V-501 (I\textsubscript{3}) as the CTA/initiator system for polymerization directly in aqueous solution. Sumerlin et al. detailed the synthesis and solution properties of diblock copolymers of DMBVA and DMA.\textsuperscript{145} MacroCTAs of both DMBVA and DMA were synthesized to examine the effect of blocking order on the efficiency of the copolymerization. The DMBVA macroCTA was prepared in water with CTP and V-501 as the CTA and initiator, respectively. After purification and isolation, the DMBVA macroCTA was then chain extended with DMA directly in water. An SEC trace of the attempted block copolymerization showed the presence of unreacted DMBVA homopolymer and higher molecular weight impurities. Alternatively, DMBVA was polymerized in the presence of a PDMA macroCTA. The SEC chromatogram of the chain extension of PDMA with DMBVA indicated near-quantitative blocking efficiency with the resulting diblock copolymers having narrow, unimodal molecular weight distributions. The differences between the blocking experiments were explained in terms
of preferred fragmentation pathways during the pre-equilibrium of the polymerization of the second monomer.

**Momomers with Zwitterionic Character**

Betaine monomers are a special class of ionic compounds which have both anionic and cationic moieties on the same residue. Donovan et al. demonstrated that 3-[2-(N-methylacrylamido)-ethyldimethylammonio]propanesulfonate (MAEDAPS) (M25), 3-[N-(2-methacroyloyethyl)-N,N-dimethylammonio]propanesulfonate (DMAPS) (M26), and 3-(N,N-dimethylvinylbenzylammonio)propanesulfonate (DMVBAPS) (M27) could be polymerized by RAFT directly in aqueous solution (0.5 M NaBr) at 70 °C using CTP (CTA1) as the RAFT CTA and V-501 (I3) as the initiating species.\textsuperscript{146} The polymerizations of all three sulfobetaine monomers were well-controlled, in good agreement with theoretical molecular weights, and produced low PDI polymers. The pseudo first-order kinetics and $M_n$ vs. conversion plots exhibit linear relationships even at extended polymerization times and high conversions. A short induction period occurs in the RAFT polymerization of these three sulfobetaine monomers. Such inhibition periods are not uncommon in polymerizations mediated by dithioesters. In a later report, Donovan and coworkers synthesized di- and triblock copolymers of DMA and MAEDAPS using CTP and a novel difunctional dithioester, respectively, and investigated the salt-responsive dissociation of the block copolymers in water.\textsuperscript{147}
Figure I-9. Betaine monomers polymerized by RAFT.

Block polymerization of another sulfobetaine was reported by Arotcarena et al. in which a PNIPAM macroCTA was chain-extended with 3-[N-(3-methacrylamidopropyl)-N,N-dimethyl]ammoniopropane sulfonate (SPP) (M24) in methanol. No data supporting the controlled polymerization of SPP was given due to difficulties in the characterization of the block copolymers. The block copolymers exhibited two thermal transitions in water in agreement with the LCST of PNIPAM and the UCST of PSPP. Virtanen et al. investigated the solutions properties of P(NIPAM-b-SPP) in more detail in a subsequent report. Additional examples of the polymerization of sulfobetaines include work performed by Morishima and coworkers, You et al., and Wang et al.

In addition to sulfobetaine monomers, the RAFT polymerization of phosphobetaine monomers has been reported by several groups. Stenzel and coworkers have studied the polymerization of 2-acryloyloxyethyl phosphorylcholine (APC) (M28) from PS and PBA macroCTAs and the application of the resulting diblock
copolymers to form biomimetic porous films and nanocontainers, respectively. The RAFT polymerization of the corresponding methyl-substituted monomer, 2-methacryloyloxyethyl phosphorylcholine (MPC) (M29), was detailed by Yusa and coworkers.\textsuperscript{154} MPC was polymerized directly in water at 70 °C using CTP and AIBN as the CTA and initiator, respectively. The rate of polymerization of MPC in water was quite fast with 90 % conversion being reached in 60 min. As for other polymerizations mediated by dithioesters, a short induction period of ~ 10 min was observed. The pseudo first-order kinetic and the $M_n$ vs. conversion plots are linear suggesting a well-behaved polymerization. A PMPC\textsubscript{96} macroCTA was then utilized in the polymerization of n-butyl methacrylate (BMA) to form amphiphilic block copolymers.

MPC has also been polymerized by Iwasaki and coworkers using the RAFT technique.\textsuperscript{155} Hydroxy-terminated poly(vinylmethylsiloxane-co-dimethylsiloxane)s (PVDMS) were functionalized with CTP via a carbodiimide coupling reaction. The difunctional macroCTAs, CTP–PVDMS–CTP, were then used to mediate the RAFT polymerization of MPC to form biocompatible triblock copolymers. Both the kinetic plots and the molecular weight evolution with conversion exhibit linear relationships for the polymerization of MPC from three CTP–PVDMS–CTP macroCTAs as expected for a well-controlled RAFT polymerization. The block copolymers were coated on PDMS and chemically bonded via hydrosilylation, which improved the surface wettability as well as reduced platelet adhesion and protein adsorption.
Aqueous Self-Assembly of Stimuli-Responsive Block Copolymers Synthesized by RAFT

Amphiphilic block copolymers spontaneously self-assemble through the association of an insoluble segment(s) of the chain when a copolymer is dissolved in a solvent system that only solvates a portion of the overall chain. For self-assembly process to occur, the block copolymer must be present at a concentration above the critical aggregation concentration (CAC), commonly called the critical micelle concentration (CMC) for micellar block copolymer systems. Below the CAC, the block copolymers exist as molecularly dissolved unimers, whereas above the CAC, the block copolymers exist as self-assembled aggregates in dynamic equilibrium with unimers. Experimentally, micelle formation is typically accomplished in either of two ways. The first involves dissolution of a block copolymer in a good solvent for all blocks followed by the gradual addition of a non-solvent for one of the blocks via dialysis. The second method involves dissolving stimuli-responsive block copolymers directly in water, and by changes induced by an external stimulus (temperature, pH, etc.), one of the blocks is rendered hydrophobic which causes the block copolymer to aggregate.

Once assembled, block copolymer aggregates can be characterized by the following parameters:

1) the equilibrium constant between aggregates and unimers
2) the CAC and critical aggregation temperature (CAT)
3) the self-assembled morphology
4) the molecular weight of the block copolymer aggregates, $M_a$
5) the aggregation number of the block copolymer assembly
6) the radius of gyration \( (R_g) \)

7) the radius of hydration \( (R_h) \)

8) the shape factor \( (R_g/R_h) \)

These characteristics of block copolymer aggregates can be determined utilizing several methods. The CAC and CAT are typically measured via scattering, fluorescence, or dye solubilization. Due to the very low CAC’s of block copolymer systems compared to those of small molecular surfactants, equilibrium conditions are only achieved after extended time periods. As such, fluorescence techniques are the preferred method for CAC and CAT determinations for self-assembling block copolymers.\(^\text{156}\)

Most reports on the self-assembly of block copolymer involve formation of spherical micelles.\(^\text{157}\) Micelles are not, however, the only structures formed from self-assembling amphiphilic block copolymers; rather, they are part of a morphological continuum that includes worm-like micelles and polymeric vesicles (commonly referred to as polymersomes in comparison to the liposomes).\(^\text{158-160}\) The observed morphologies are a result of the inherent molecular curvature and the resulting packing of the block copolymer chains. The packing of the polymer chains, which is influenced by a number of factors including molecular weight, polymer composition, polydispersity, and chain architecture,\(^\text{161}\) can be described by the packing parameter, \( p \), which is defined as:

\[
p = \frac{v}{a_h l_c}
\]

(1)

where \( v \) is the volume of the hydrophobic chains, \( a_h \) is the optimal head group area, and \( l_c \) is the length of the hydrophobic tail. The value of \( p \) is often used to predict which morphology is favored (spherical micelles when \( p \leq 1/3 \), cylindrical micelles when \( 1/3 \leq \))
\( p \leq 1/2 \), and vesicles (polymersomes) when \( 1/2 \leq p \leq 1 \). More recently, Discher and Eisenberg developed an empirical relationship (Figure I-10) between the block copolymer composition and the self-assembled morphologies. Spherical micelles are expected for polymers with hydrophilic mass fractions \( (f) \) greater than 45 %, while copolymers with \( f \approx 35 \pm 10 \% \) typically assemble into polymersomes. There have, however, been examples where spherical micelles are formed at \( f < 50 \% \). These occurrences have been attributed to the ability of the hydrophilic block to balance the disproportionately large hydrophobic block. A number of reviews have been published describing the correlation between the hydrophilic mass fraction and the resulting solution morphology.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure10.png}
\caption{Schematic representation of the empirical relationship between hydrophilic mass fraction and solution morphology as proposed by Discher and Eisenberg.}
\end{figure}

The size and molecular weight of the block copolymer aggregates are determined using light scattering techniques. The radius of hydration is generally determined using dynamic light scattering (DLS). By measuring the translational diffusion coefficient \( (D_{\text{app}}) \) of the aggregates, the hydrodynamic radius can be determined using the Stokes-
Einstein equation (Equation 6) where $k_B$ is the Boltzmann constant, $T$ is the temperature, and $\eta$ is the viscosity of the medium.

$$R_h = \frac{k_B T}{6\pi \eta D_{app}}$$

Similarly, the radius of gyration ($R_g$) of block copolymer assemblies is determined by static light scattering (SLS). The $R_g$ is determined from the slope of either a Zimm plot of the scattering intensity ($I_{ex}$) versus the square of the scattering vector ($q^2$) or a Berry plot ($I_{ex}^{-1/2}$ vs. $q^2$) in instances where a Zimm treatment results in curvature of the data due to the formation of large particles ($\geq 100$ nm). SLS is also useful in determination of the aggregate molecular weight, $M_a$, and the aggregation number.

*Self-Assembly of Thermally-Responsive Block Copolymers*

Block copolymers composed of thermo-responsive PNIPAM and various hydrophilic blocks have been widely studied. For example, Convertine in our group described the synthesis of thermally responsive di- and triblock copolymers of DMA and NIPAM at room temperature. The AB and ABA block copolymers were prepared with fixed PDMA but variable PNIPAM block lengths, so as to facilitate the systematic evaluation of the effect of the DP of PNIPAM on the aqueous solution properties. To demonstrate this self-assembly process, as well as the reversibility of this process, DLS was utilized to measure the $D_h$ for the diblock copolymer $P($NIPAM$_{460}$-$b$-DMA$_{100}$) at 25 and 45 °C. At 25 °C the $D_h$ was ~ 10 nm which is consistent with molecularly dissolved unimer chains, whereas at 45 °C, above the LCST of PNIPAM, the $D_h$ is ~ 80 nm, and corresponds to an aggregation number of ~ 213 as determined by SLS. The reversibility of the self-assembly process was demonstrated by monitoring the changes in $D_h$ through 5 heating/cooling cycles.
Lokitz et al. in our group synthesized a micelle-forming block copolymer, P(DMA-b-[NIPAM-stat-AVAL]) utilizing aqueous RAFT polymerization. A series of block copolymers were synthesized by employing PDMA as a macro-CTA to mediate the statistical copolymerization of NIPAM and AVAL. DLS measurements demonstrated that the CAT for the block polymers could be tuned to range from ~10 to 36 °C by adjusting the solution pH. Micelles with apparent hydrodynamic diameters from 45 to 86 nm were formed between pH 2 and 5. Above pH 5, a sufficient number of the AVAL units were ionized to prevent micellization.

De et al. employed an azido-functionalized CTA to mediate the RAFT polymerization of NIPAM and DMA. The resultant α-azido terminal diblock copolymers were coupled with propargyl folate via Cu(I)-catalyzed azide-alkyne cycloaddition to form temperature-responsive bioconjugates. DLS demonstrated that the block copolymer underwent self-assembly at 34 °C to yield aggregates of 46 nm. The thermo-induced assembly resulted in aggregates capable of controlled release of a model hydrophobic drug, dipyridamole (DIP). The block copolymer aggregates dissociated to unimers to yield a burst release of DIP at 25 °C. The release of DIP from the self-assembled aggregates at 37 °C was found to be much slower.

In 2007, An et al. reported a RAFT precipitation polymerization of NIPAM using PDMA-based macro-CTA. When the thermoresponsive PNIPAM blocks were sufficiently long, the chains collapsed to form micellar aggregates stabilized by the water-soluble PDMA blocks. When the cross-linker N,N’-methylenebisacrylamide (BIS) was incorporated, the growing polymers were crosslinked and would swell upon cooling due to the influx of water into the nanoparticle core. In the absence of crosslinker, the
nanoparticles dissociated into double hydrophilic block copolymers due to the hydrophobic-to-hydrophilic transition of the PNIPAM block when the solution was cooled below the LCST.

The preparation of a $\text{P(NIPAM}_{242}\text{-b-DMA}_{242}\text{-b-NIPAM}_{242}}$ BAB triblock copolymer was recently reported by Skrabania et al. These BAB triblock copolymers in a 0.1 wt% aqueous solution self-assembled into micelles with $D_h \sim 100$ nm above the LCST of PNIPAM at 0.1 wt%. However, according to work by Kirkland in our group, $\text{P(NIPAM}_{455}\text{-b-DMA}_{277}\text{-b-NIPAM}_{455}}$ BAB triblock copolymer formed thermo-reversible gels at concentrations above 7.5 wt% and above the phase transition temperature of PNIPAM.

Other permanently hydrophilic monomers have been copolymerized with NIPAM to synthesize thermo-responsive block copolymers. Yan et al. reported the synthesis of $\text{P(EO}\text{-b-NIPAM})$ using a PEO macro-CTA. The diblock copolymer formed large, loose structures at temperatures between 28 and 42 °C prior to collapse of the PNIPAM block leading to micellization. The size and proportion of these loose structures decreased with increasing concentration as driven by the incompatibility of the two blocks. Another $\text{P(EO}\text{-b-NIPAM})$ example was recently reported by You et al.

Block copolymers comprised of PNIPAM and hydrophobic blocks have also been investigated for self-assembly behavior in aqueous solution. For example, Zhu et al. reported the synthesis of a diblock copolymer, $\text{poly(NIPAM-b-[2-hydroxyethyl methacrylate-b-ε-caprolactone]_n})$ ($\text{P(NIPAM-b-[HEMA-b-PCL]_n})$ (n = 3 or 9)), by sequential RAFT polymerization of NIPAM and a HEMA-b-PCL macromonomer. The copolymer which contains a thermo-responsive PNIPAM block and a biodegradable
hydrophobic P(HEMA-b-PCL) block self-assembles into micelles in water at room temperature. Transmission electron microscopy (TEM) micrographs show a spherical morphology with a size range of 30 - 100 nm for P(NIPAM-b-[HEMA-PCL])₃. The LCSTs of the copolymers are both around 36 °C. The controlled drug release from P(NIPAM-b-[HEMA-PCL])₃ micelles was examined at different temperatures (below and above LCST) using paclitaxel as a model drug compound.

Zhang et al. prepared poly(NIPAM-b-γ-methacryloxypropyltrimethoxysilane) (P(NIPAM-b-MPS)) via RAFT polymerization in 1,4-dioxane. In aqueous solution, amphiphilic P(NIPAM-b-MPS) self-assembled into micelles with PMPS core and PNIPAM shell. The diameters of the resultant spherical nanoparticles were 40-60 for P(NIPAM₄₈-b-MPS₆₀) and 20-40 nm for P(NIPAM₃₀₀-b-MPS₅₂). A base-catalyzed sol-gel process inside PMPS core resulted in PNIPAM-encapsulated silica hybrid core-shell nanoparticles. TEM, DLS and SLS studies revealed monodisperse hybrid nanoparticles with densely grafted PNIPAM brush at the surface of silica core. For the nanoparticles prepared from P(NIPAM₃₀₀-b-MPS₅₂), the average R₉ was 72 nm at 20 °C and decreased to 56 nm at 26 °C. A small plateau was reached in the range 26-30 °C. Above 31 °C, R₉ further decreased from 55 to 49 nm in the temperature range 31-36 °C. This indicated a thermo-responsive two-stage collapse of the grafted PNIPAM brush upon heating. Hybrid nanoparticles prepared from PDMAEMA-b-PNIPAM block copolymers have also been investigated by the same group.

Tang et al. reported the synthesis of P(MMA-b-NIPAM) via RAFT polymerization. The copolymers formed well-defined micelles in dilute solution at low temperature as probed by DLS and small angle neutron scattering (SANS), which
indicated that the copolymer micelles below 31 °C were composed of small PMMA cores and large coronas of PNIPAM chains. The SANS data indicated formation of micelles with a very small core to corona ratio that behaved like star polymers with a large number of arms. Upon heating above 31 °C, dehydration of PNIPAM led to an increase in micelle size. Rheology was used to measure the dynamic shear moduli of gels at low temperature and to locate a phase transition boundary consistent with the LCST observed visually and by DLS.

Zhou et al. reported the preparation of triblock copolymers of poly(stearyl methacrylate-b-NIPAM-b-stearyl methacrylate) (P(SMA-b-NIPAM-b-SMA)) with varying molecular weights. By changing the organic solvent and adjusting the copolymer composition, multiple morphologies, including vesicles, core-shell spherical aggregates, and pearl-necklace-like aggregates were obtained. The aggregates also showed thermo-responsive and pH-responsive properties through the LCST of PNIPAM and the two carboxyl end groups of the copolymer. For P(SMA10-b-NIPAM68-b-SMA10), at a polymer concentration of 0.1 wt % in tetrahydrofuran (THF)/water 70/30 (w/w), the diameter of the aggregates increased from ca. 800 nm to ca. 1550 nm above the LCST of the PNIPAM. This can be attributed to the association of several small aggregates since the PNIPAM shells are hydrophobic above LCST. Moreover, the triblock copolymer formed giant spheres with average diameters of 1600 nm at pH 2.0, 960 nm at pH 5.4 and 1200 nm at pH 9.0, respectively. This indicated that the hydrophilicity of the carboxyl end groups at different solution pH values affected aggregation behavior.

Another hydrophobic-b-(thermo-responsive) block copolymer example has been reported by Walther et al. A series of triblock copolymers was synthesized composed
of a hydrophilic PEO block and a hydrophobic PBA block, with thermo-responsive PNIPAM or PDEA or with permanently hydrophilic PAM or PHPMA blocks. The hydrophilic-to-hydrophobic balance was varied by changing the third block and/or changing an environmental stimulus. For example, the triblock copolymer P(EO$_{114}$-b-nBuA$_{250}$-b-DEA$_{135}$) formed different morphologies depending on the solution conditions as determined by cryogenic transmission electron microscopy (cryo-TEM). Direct dissolution of this copolymer into water led to a large fraction of vesicles surrounded by a corona of PEO or PDEA and a small fraction of branched wormlike aggregates. By contrast, dialysis from dioxane into water led to spherical micelles and wormlike aggregates. This suggested that direct dissolution favored the generation of aggregates of lower curvature. A subsequent heating/cooling cycle of the spherical micelles and wormlike aggregates to 45 °C and back to room temperature led to the formation of worm-like aggregates and the disappearance of spherical micelles.

Other thermally-responsive block copolymers formed from PNIPAM and a hydrophobic block have been investigated including poly(lactide-$b$-NIPAM-$b$-lactide)$^{177}$, P(NIPAM-$b$-oligofluorene(OF)-$b$-NIPAM)$^{178}$, chiral amphiphilic poly(6-O-p-vinylbenzyl-1,2:3,4-Di-O-isopropylidene-D-galactopyranose-$b$-NIPAM) and poly(20-(hydroxymethyl)-pregna-1,4-dien-3-one methacrylate-$b$-NIPAM)$^{179}$, and poly(D,L-lactide-$b$-[NIPAM-co-DMA]).$^{180}$

**Self-Assembly of pH-Responsive Block Copolymers**

Mitsukami et al. synthesized a series of block copolymers composed of a fixed VBTMAC block and varying lengths of an DMVBA block (DPs ranging from 11 to 50) via RAFT polymerization.$^{181}$ The pH-dependent micellization behavior was followed by
potentiometric titration, $^1$H NMR spectroscopy, DLS, SLS, and fluorescence techniques. At pH < 5.5, the PDMVBA block is fully protonated, and hence the block copolymers act as simple polyelectrolytes. Above pH 7, the PDMVBA block becomes deprotonated and the block copolymers aggregate into micelles. Light scattering and fluorescence measurements indicated that the number of polymer chains comprising one micelle (i.e. $N_{agg}$) increased from 3 to 12 as the DP of DMVBA increased from 11 to 50 at pH 10.0. However, for the random copolymer with a DMVBA/VBTMAC molar ratio of 57:53, unimolecular micelles ($N_{agg}$ ≈ 1) were formed at pH 10.0.

Using RAFT polymerization, Lowe et al. synthesized homo- and copolymers of phosphonium-based styrenic monomers, TMP and TPP, and VBA in aqueous media. $^{121}$ $^{13}$C NMR spectroscopy was utilized to study the pH-responsive behavior of the block polyampholytes. At pH 10.0, the C=O resonance associated with the carboxylate is clearly evident in spectrum A at $\delta = 175$ ppm when the PVBA residues are expected to be ionized and hence hydrophilic and solvated. By contrast, at pH 2.0 (B), when the PVBA residues are fully protonated, the C=O resonance is not observed or completely absent. Additionally, changing the solution pH from 10.0 to 2.0 results in a broadening of the resonances associated with the aromatic carbons. These features are entirely consistent with a hydrophilic to hydrophobic phase transition of the PVBA block. Subsequently, Lowe’s group reported the synthesis and self-assembly behavior of diblock copolymers of TMP and DMBVA in aqueous solution. $^{182}$ Using a combination of DLS, NMR, and fluorescence spectroscopies, the diblock copolymers were shown to undergo pH-induced self-assembly, presumably forming core-shell polymeric micellar structures with the PDMBVA block forming the hydrophobic aggregate core at high pH, stabilized by the
hydrophilic TMP corona. Such aggregation was also shown to be completely reversible dependent on solution pH.

*Self-Assembly of Block Copolymers Responsive to Other Stimuli*

Recently, Vijayakrishna et al. copolymerized three imidazolium-based ionic liquid (IL) monomers, namely, 3-(1-ethyl imidazolium-3-yl)propylmethacrylamido bromide, 2-(1-methylimidazolium-3-yl)ethyl methacrylate bromide, and 2-(1-ethylimidazolium-3-yl)ethyl methacrylate bromide with MAA by the RAFT process in methanolic solutions at 70 °C. The resultant diblock copolymers could be further manipulated and made to self-assemble into micelle-like structures in water by exchanging the bromide (Br-) counteranion of IL blocks for -N(SO$_2$CF$_3$)$_2$ (Fig. 46). This anion exchange induced a transition from hydrophilic to hydrophobic as verified by the immiscibility of the PILs in water. With the salt-responsive switch in hydrophilicity of the PIL blocks, the PMAA-$b$-PIL copolymers form water-soluble micellar aggregates stabilized by a PMAA shell.

Recently, Sumerlin’s group prepared the water-soluble boronic acid copolymer poly(4-vinylphenylboronic acid-$b$-DMA) using RAFT polymerization. Later, the same group reported the synthesis of block copolymer poly(3-acrylamidophenylboronic acid-$b$-DMA) (P(APBA-$b$-DMA)). Boronic acids are sensitive to both pH and solution diol concentration. In aqueous media, boronic acids exist in equilibrium between forms that are neutral (typically insoluble) and anionic (soluble). Boronate esters are readily formed in the presence of vicinal diols. An increase in the concentration of boronate ester shifts the ionization equilibria, effectively lowering the pK$_a$ of the acid. Thus, complexation adjusts the overall equilibrium from neutral/insoluble boronic acid moieties to
anionic/hydrophilic boronates. Therefore, the extent of ionization (and water solubility) of boronic acid-containing polymers increases with diol concentration. P(APBA<sub>131</sub>-b-DMA<sub>138</sub>) was dissolved at pH 10.7 to give unimers with a D<sub>h</sub> of approximately 7 nm. When the pH was reduced below the pK<sub>a</sub> of the PAPBA block (pK<sub>a</sub> ≈ 9), aggregates with an average hydrodynamic diameter of 35 nm were observed by DLS. The authors assumed that the aggregates were micelles composed of a hydrophilic PDMA corona and a hydrophobic PAPBA core. As mentioned above, P(APBA-b-DMA) is also responsive to the concentration of diols. Upon the addition of glucose, the D<sub>h</sub> dramatically decreased to 9 nm, indicative of aggregation disassembly. Thus, the diblock copolymer of APBA and DMA showed both pH- and sugar-responsive behavior.

Self-Assembly of Copolymers Comprised of Two Responsive Blocks

Copolymers bearing two blocks which respond to different stimuli can exhibit “schizophrenic” micellization behavior. In 2004, Schilli et al. found that well-defined PNIPAM-b-PAA copolymers form micelles or other aggregates depending on solvent, temperature, pH and block lengths. The solubility of the PAA block in aqueous solution depends on the pH of the medium. The lower the pH, the more carboxylate groups of the PAA blocks are protonated, and the less soluble this block becomes in aqueous media. At pH 4.8 virtually all carboxylate groups are ionized and the PAA segment is readily soluble in water. The cloud point (CP) is raised from 29 °C at pH 4.5 to about 35 °C at pH 5-7 for P(NIPAM<sub>50</sub>-b-AA<sub>110</sub>). Thus, the LCST of PNIPAM is altered through the attachment of AA chains, increasing if the PAA block is deprotonated and hydrophilic and decreasing if the PAA block is protonated and hydrophobic.
Kulkarni et al. synthesized a biotin-terminated P(NIPAM-b-AA) and investigated the thermally induced aggregation behavior. The CP and aggregation properties of the biotinylated diblock copolymer were also shown to be dependent on pH. At pH 7.0 and temperatures above the LCST, the block copolymer was found to form particles of ~ 60 nm while at pH 5.5 and 20 °C, the copolymer formed large aggregates (ca. 218 nm), presumably driven by hydrogen bonding between the -COOH groups of PAA with other -COOH groups and with the -CONH- groups of PNIPAM. When the pH was lowered to 4.0, large particles were formed above and below the LCST (ca. 700 and 540 nm, respectively).

Liu’s group reported a dually-responsive diblock copolymer, P(NIPAM-b-DEAEMA), which was synthesized via RAFT polymerization. The diblock copolymer exhibits intriguing “schizophrenic” micellization behavior in aqueous solution, forming PDEAEMA-core micelles at alkaline pH and room temperature and PNIPAM-core micelles at acidic pH and elevated temperature. A similar diblock copolymer has also been prepared via RAFT polymerization and reported by Liu et al. UV-vis spectrophotometry showed that the LCST of P(DEAEMA-b-NIPAM) decreased with increasing solution pH due to deprotonation of PDEAEMA block.

Liu’s group has also utilized RAFT polymerization to synthesize a sulfobetaine block copolymer, poly(N-(morpholino)ethyl methacrylate-b-4-(2-sulfoethyl)-1-(4-vinylbenzyl)pyridinium betaine) (P(MEMA-b-SVBP)), capable of purely salt-responsive “schizophrenic” micellization behavior in aqueous solution. In aqueous solution, the PMEMA block becomes insoluble in the presence of Na₂SO₄ (>0.6 M), whereas PSVBP molecularly dissolves in the presence of NaBr (>0.2 M). Thus, the diblock copolymer can
form either PMEMA-core or PSVBP-core micelles, depending on the concentration and type of added salts. The equilibrium structures of these two types of micelles were characterized via a combination of $^1$H NMR and laser light scattering (LLS). The kinetics of salt-induced formation/dissociation of PMEMA-core and PSVBP-core micelles and the structural inversion between them were investigated by employing a stopped-flow, light scattering technique. In the presence of 0.5 M NaBr, the addition of Na$_2$SO$_4$ (> 0.6 M) induces the formation of PMEMA-core micelles stabilized with well-solvated PSVBP coronas. The structural inversion from PMEMA-core to PSVBP-core micelles proceeds first with the dissociation of PMEMA-core micelles into unimers, followed by the formation of PSVBP-core micelles. On the other hand, the PSVBP-core to PMEMA-core process exhibits different kinetic sequences. Immediately after the salt jump, PMEMA corona chains are rendered insoluble, and unstable PSVBP-core micelles undergo intermicellar fusion; this is accompanied and/or followed by the solvation of PSVBP cores and structural inversion into colloidally stable PMEMA-core micelles.

Lowe et al. synthesized a diblock copolymer comprised of NIPAM and VBA.\textsuperscript{122} The diblock copolymer of NIPAM and VBA also exhibited “schizophrenic” micellization by taking advantage of the stimuli responsive characteristics of both blocks. Specifically, raising the temperature to 50 °C, while at pH 12 results in supramolecular self-assembly to yield nanosized species ($D_h = 51.0$ nm) that are composed of a hydrophobic PNIPAM-core stabilized by a hydrophilic PVBC corona. Conversely, lowering the solution pH to 2.0 at ambient temperature results in the formation of aggregates ($D_h = 66.7$ nm) in which the PVBA block is now hydrophobic and in the core, stabilized by the hydrophilic NIPAM block.
Xu et al. reported the synthesis of a triblock copolymer P(EO-b-DMAEMA-b-NIPAM) which contains both a pH-responsive PDMAEMA block and a thermo-responsive PNIPAM block. The DLS data demonstrated that uniform micelles (the size was dependent on the composition of triblock copolymer) were formed in aqueous media above the LCST of PNIPAM block. The hydrodynamic diameter was also dependent on the pH value of the solution due to the pH-responsive PDMAEMA middle block.

Combing ring-opening polymerization and RAFT polymerization, Zhang et al. prepared a diblock copolymer, poly(L-glutamic acid-b-NIPAM) (P(LGA-b-NIPAM)). The thermo-induced self-assembly and pH-responsive aggregation were investigated by $^1$H NMR, DLS and TEM. Aggregates of different morphologies, including huge tree-like (size up to 6-8 μm), interconnected spherical (30-50 nm), and fiber-like (cylinder shaped) aggregates formed from dilute aqueous solution of the block copolypeptides at 50 °C with solution pH values of 8.0, 9.0, and 10.0, respectively. Deng et al. also synthesized P(NIPAM-b-LGA) using sequential RAFT polymerization and ring-opening polymerization (ROP). At pH 3 and 25 °C, the diblock copolymer formed PLGA-core micelles with $D_h$ of 40-60 nm. While at pH 10 and 45 °C, the diblock copolymer formed PNIPAM-core aggregations. $R_g$ and the $R_h$ were determined to be 164 nm and 102.1 nm, respectively, resulting in a $R_g/R_h$ of 1.61. This revealed that such aggregation should be rod-like.

Shell Cross-linked Nanoassemblies

It is well known that block copolymer assemblies can be used as drug delivery vehicles. However, certain limitations of self-assembled nanostructures preclude the
realization of their use in practical applications. One major limitation is the dilution-induced dissociation of the amphiphilic nanostructure into unimers after administration \textit{in vivo}. When the copolymer concentration falls below the CAC, as it does when administered to a patient, the nanostructure dissociates, resulting in the premature release of the active compound. To address the stability issue of amphiphilic block copolymer micelles, shell cross-linking (SCL) approaches, originally reported by Wooley et al.\textsuperscript{188}, have been developed.

In their initial report, Wooley and coworkers utilized diblock copolymers of polystyrene and 4-(chloromethyl)styrene-quaternized poly(4-vinylpyridine) (QP4VP).\textsuperscript{188} Shell cross-linking was accomplished by radical oligomerization of the pendant styrenyl functionalities in the P4VP corona. A number of alternative chemistries have been developed to accomplish the shell cross-linking of polymeric nanostructures directly in water. Ding and Liu reported the synthesis of pH-responsive SCL micelles from an ABC triblock copolymer bearing photocross-linkable cinnamoyl groups.\textsuperscript{189} Alternatively, a number of approaches have been developed to utilize low molecular weight cross-linkers. Armes’ group has reported numerous examples of shell cross-linking of stimuli-responsive micelles through the quaternization of tertiary-amines using bis(2-iodoethoxy)ethane (BIEE)\textsuperscript{190-196} as well as utilizing a Michael addition between divinyl sulfone (DVS) and pendant hydroxyl functionalities.\textsuperscript{197} While both of these could be accomplished under mild conditions, these reagents are mutagenic, and an alternative strategy was desired. As such, the Armes’ group subsequently developed polyelectrolyte complexation for ionic cross-linking micelles possessing a charged shell.\textsuperscript{198} Polyelectrolyte complexation offers several advantages over the previously discussed
cross-linking methods: (1) most polyelectrolytes exhibit low toxicity; (2) physical cross-linking is relatively fast; (3) aside from the freed counterions, no small-molecule side products are released; (4) ionic cross-linking can be readily reversed by the addition of salt.\textsuperscript{199}

Since the early work by Armes and coworkers\textsuperscript{198}, our group has investigated a number of stimuli-responsive copolymer systems capable of forming SCL micelles and vesicles through interpolyelectrolyte complex (IPEC) formation. In 2006, Li and coworkers reported the formation of vesicles prepared from the self-assembly of P(APMA-\text{-}b\text{-}NIPAM) in water.\textsuperscript{43} At room temperature, the diblock copolymer readily dissolves in aqueous solution; however, upon increasing the solution temperature above the LCST of the PNIPAM block, the diblock copolymer self-assembles into uniform vesicles with hydrodynamic diameters of approximately 280 nm. Since APMA is pH-responsive, the vesicle stability was investigated at varying pH values. The vesicles remained intact over the studied pH range while the size varied with the degree of protonation of the APMA units (310 nm at pH 3.0 and 220 nm at pH 10.8). The cationic PAPMA shells of the vesicles were subsequently cross-linked through IPEC formation with an anionic polyelectrolyte, PAMPS. After shell cross-linking, the size of the vesicles decreased from 270 to 140 nm due to the charge neutralization of the shell. Successful cross-linking was demonstrated as the vesicles remained intact at low temperatures. The resulting cross-linked vesicles were stable over a wide pH range and moderate electrolyte concentration. The cross-linking could be reversed by increasing the electrolyte concentration to 0.8 M NaCl.
Lokitz et al. demonstrated the successful shell cross-linking of block copolymers derived from amino acid based monomers. Tri- and pentablock copolymers of N-acryloyl alanine (AAL), NIPAM, and DMA reversibly self-assemble into PNIPAM-core micelles in response to changes in temperature. The presence of the anionic carboxylate groups in the PAAL shell makes such a system amenable to shell cross-linking through IPEC formation. Addition of an equimolar amount of cationic PVBTAC led to the SCL micelles. The reversibility of the electrostatically cross-linked micelles was investigated by introducing simple salts. The cross-linked micelles remain intact in aqueous solutions with NaCl concentrations as high as 0.3 M. At 0.4 M NaCl concentration, however, the SCL micelles dissociate to unimers, demonstrating the reversible nature of the IPEC shell. Interestingly, above 0.8 M NaCl, aggregates reform as the PNIPAM blocks are “salted out” of solution.

The reaction of a difunctional amine with an activated ester moiety incorporated in the shell of nanoassemblies provides a facile and efficient method for the formation of SCL nanoassemblies. Recently, Li and coworkers reported the synthesis of P(EO-b-(DMA-stat-N-acryloxsuccinimide (NAS))-b-PNIPAM which undergoes thermally-responsive self-assembly into micelles. The NAS moieties, located in the shell of the micelles, were subsequently reacted with ethylene diamine to cross-link micelle coronas. This reaction proceeds rapidly, reaching over 95 % completion in 2 h. The aggregate structure of the SCL micelles is conserved after reducing the solution temperature below the LCST as confirmed by DLS and atomic force microscopy (AFM).

While reacting the NAS moiety with ethylene diamine proved to be a facile method for producing SCL micelles, the cross-linking reaction is not reversible. The use
of a cleavable functionality, however, should allow the breakdown of SCL micelles and subsequent dissociation to unimers \textit{in situ}. To demonstrate the feasibility of such a process, a micelle-forming triblock copolymer, P(EO_{45}-(DMA_{98}-stat-NAS_{30})-b-NIPAM_{87}), was synthesized by RAFT polymerization.\textsuperscript{202} After heating a solution of the block copolymer above the LCST, the micelles were cross-linked with cystamine, a disulfide-containing diamine. The resulting disulfide cross-links were then cleaved through chemical reduction by either a thiol exchange reaction with dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine hydrochloride (TCEP). Using either reagent leads to dissociation of the SCL micelles into unimers as confirmed by DLS. After removal of the excess reducing agent, addition of cystamine results in the reformation of the SCL micelles through a thiol/disulfide exchange reaction.

Our most recent report utilizing an activated ester for the formation of SCL micelles details the use of a cleavable, temperature-responsive polymeric crosslinker.\textsuperscript{44} In this study, micellization of the pH-responsive triblock copolymer, P(EO-b-APMA-b-DPAEMA), was induced by increasing the solution pH above 6.0, thus rendering the PDPAEMA block hydrophobic. To produce the polymeric cross-linking agent, the RAFT polymerization of NIPAM was mediated by CMP. The end groups were subsequently functionalized with an activated ester via carbodiimide coupling to give (α,ω-N-hydroxysuccinimidyl ester)-PNIPAM (NHS-PNIPAM-NHS). The primary amine functionality in the PAPMA shell was then reacted with the temperature-responsive cross-linking agent. The SCL micelles were both pH- and temperature-responsive and because the polymeric cross-linking agent contains a trithiocarbonate core, the cross-links can be cleaved easily to allow dissociation of the micelles to unimers. Using the same
pH-responsive triblock copolymer, we achieved the “one-pot” synthesis of reversible SCL micelles. A water-soluble, reversibly cleavable crosslinker, dimethyl 3,3’-dithiobispropionimidate (DTBP), was employed to “lock” the P(EO-b-APMA-b-DPAEMA) micelles. The disulfide-containing cross-linker provides a reversibly cleavable site in the SCL micelles; DTT was used as a cleaving agent while SCL micelles were reformed under oxidizing conditions.

Gold Nanoparticles

Gold nanoparticles (AuNPs) have been the focus of intense research for a number of years due to their potential applications as biosensors and catalysts as well as in optical, electronic, and magnetic devices. This growing interest is a result of the unique chemical and physical properties inherent to AuNPs. A unique characteristic of AuNPs is the presence of the surface plasmon band (SPB) which, according to Mie theory, is attributed to the dipole oscillations of the free electrons in the conduction band. The SPB of AuNPs appears as a broad absorption band in the visible light region around 520 nm. There are several factors that affect the SPB, one of which is the particle size. The maximum absorption experiences a red shift toward higher wavelengths with increasing size of the AuNPs. For example, AuNPs with average diameters of 9 and 99 nm exhibit a maximum absorption at 517 and 575 nm respectively with intermediate sizes falling within these values. The SPB can also experience shift in the maximum absorption wavelength based on the refractive index of the solvent as predicted by Mie theory. This phenomenon is of great interest in the areas of biophotonics and materials science.
AuNPs are synthesized via a number of methods. Prior to 1994 AuNPs were largely produced by the citrate reduction of a metal salt precursor. In 1994, the Brust-Schiffrin method for Au nanoparticles in a two phase system was published and has since been adapted to produce other AuNPs using alkanethiol ligands. The Burst-Schiffrin method allows for the facile synthesis of stable AuNPs with narrow dispersities and controllable sizes. In 1995, Brust et al. modified their method into a one phase system to allow the use of a variety of thiol functionalized ligands. In these studies it was found that larger thiol/gold ratios yield smaller average core sizes. Experimenters also noted that the fast addition of the reducing agent and the use of cooled solutions led to smaller, monodisperse TMNPs. This work was extended by the McCormick research group to include the use of well-defined polymers and copolymers synthesized via the RAFT process as stabilizing agents. By mixing transition metal salt precursors with dithioester-terminated polymers and copolymers, Lowe et al. were able to reduce the transition metal salt and the dithioester group to form TMNPs with average diameters of 5-10 nm (Scheme I-4). Recently, there have been numerous reports utilizing amine-containing copolymers that can act as both a reducing agent and a stabilizing agent. Ishii and coworkers reported the synthesis of biotin-functionalized PEGylated gold nanoparticles using a PDMAEMA block to reduce AuCl₄⁻ to zero-valent AuNPs. Armes and coworkers recently reported the synthesis of poly(2-(methacyrloyloxy)ethyl phosphorylcholine)-coated AuNPs also using PDMAEMA as a reducing block. Such systems where a polymeric amine acts as the reductant are advantageous due to the lack of toxic boride contaminants when NaBH₄ is used as the reducing agent. As the ability
to specifically tailor the stabilizing ligands around AuNPs improves, the use of AuNPs for biorelevant applications has continued to be an active area of research.\textsuperscript{218}

\textbf{Scheme I-4.} Synthesis of TMNPs using a dithioester-terminated polymer synthesized by the RAFT free radical polymerization process.
CHAPTER II

OBJECTIVES OF RESEARCH

Reversible addition–fragmentation chain transfer (RAFT) is arguably the most versatile living radical polymerization technique in terms of the reaction conditions and monomer selection. Since the introduction of RAFT in 1998, the McCormick research group has employed the RAFT process to synthesize a wide range of water soluble (co)polymers with predetermined molecular weights, low polydispersions, and advanced architectures. Included in this effort is the synthesis of stimuli-responsive block copolymers for potential applications in biomedical delivery applications. While a vast amount of work has been reported on the aggregation of stimuli-responsive block copolymers into spherical micelles, relatively little work has been performed relating the assembly into other morphologies (worm-like micelles, vesicles, etc.). Our group has also invested considerable effort in developing methodologies to reversibly cross-link such self-assembled nanostructures. The overall goals of this research are to synthesize well-defined stimuli-responsive block copolymers via RAFT polymerization in order to investigate the relationship between the block copolymer composition and the resulting aqueous solution morphology and to reversibly “lock” the nanostructures through the in situ formation of gold nanoparticles (AuNPs) in the nanostructure shells.

The specific objectives of this research are as follows:

1. Synthesize a well-defined series of stimuli-responsive block copolymers of \( N \)-isopropylacrylamide (NIPAM) \( (M_1) \) and \( N,N \)-dimethylaminoethyl methacrylate (DMAEMA) \( (M_{17}) \)
2. Investigate the influence of block copolymer composition, copolymer concentration, solution pH value, and salt concentration on the temperature-induced self-assembly of P(DMAEMA-b-NIPAM)

3. Cross-link self-assembled P(DMAEMA-b-NIPAM) nanostructures via the in situ formation of AuNPs

4. Perform ligand exchange reactions with a small molecule thiol and a polymeric thiol to reverse the AuNP cross-linking of micelles and vesicles

5. Synthesize a series of diblock copolymers of N,N-diethylaminoethyl methacrylate (DEAEMA) (M19) and NIPAM specifically targeting compositions capable of assembly into micelles and vesicles

6. Investigate the “schizophrenic” aggregation behavior of two P(DEAEMA-b-NIPAM) copolymers in response to changes in temperature and solution pH

7. Characterize all (co)polymers with respect to molecular weight and copolymer compositions via size exclusion chromatography and \(^1\text{H}\) NMR

8. Characterize all self-assembled nanostructures using dynamic and static light scattering, transmission electron microscopy, and \(^1\text{H}\) NMR.

This work may be divided into four sections. The first section concerns work performed in collaboration with Dr. Yuting Li on the self-assembly of vesicles (polymersomes) from a block copolymer of DMAEMA and NIPAM and their subsequent “locking” through the in situ AuNP formation. The second section details work performed on a series of DMAEMA and NIPAM block copolymers to investigate the effect of various experimental parameters (copolymer composition and concentration, solution pH value, and ionic strength) on the temperature-induced aggregation behavior
and the resultant solution morphology. The formed nanostructures were subsequently cross-linked with AuNPs and investigated using dynamic light scattering and transmission electron microscopy. In the third section, the ability to reverse the cross-linking of the AuNP-“locked” nanostructures through the use of ligand exchange reactions is discussed. The fourth section concerns the investigation of copolymer composition of two dually-responsive block copolymers of DEAEMA and NIPAM capable of “schizophrenic” aggregation behavior.
CHAPTER III
EXPERIMENTAL

Materials

All chemicals were purchased from Aldrich at the highest available purity and were used as received unless otherwise noted. N-isopropylacrylamide (NIPAM) (M1) (97%, Aldrich) was recrystallized twice from hexane. N,N-dimethylaminoethyl methacrylate (DMAEMA) (M17) and N,N-diethylaminoethyl methacrylate (DEAEMA) (M19) were dried with CaH$_2$ and vacuum distilled prior to use. O-[2-(3-Mercaptopropionylamino)ethyl]-O’-methylpolyethylene glycol (PEG-SH, 5,000 g/mol) and cysteamine were purchased from Aldrich and used as received. 4,4-Azobis(4-cyanopentanoic acid) (V-501) (I3) and 4,4’-Azobis[2-(imidazolin-2-yl)propane] dihydrochloride (VA-044) (I3) were donated by Wako Chemicals and were recrystallized twice from methanol prior to use. 4-Cyanopentanoic acid dithiobenzoate (CTP) (CTA1) was prepared as previously reported. 4-Cyano-4-(ethylsulfanylthiocarbonyl) sulfanylpentanoic acid (CEP) (CTA8) was synthesized according to a previous literature procedure.
Figure III-1. Compounds used for the synthesis of stimuli-responsive block copolymers.

Polymerizations

*General Procedure for the RAFT Polymerization of DMAEMA*

A solution of CTP (0.0177 g, 0.0637 mmol), DMAEMA (2.00 g, 12.7 mmol), and V-501 (0.00354 g, 0.0127 mmol) in 6.5 mL of dioxane were added to a 25 mL round bottom flask sealed with a rubber septum. The solution was sparged with nitrogen for approximately 30 min and the flask was placed in a preheated oil bath at 70 °C. The reaction was terminated after 8 h (69 % conversion) by cooling the reaction tube in an ice bath followed by exposure to air. The resultant PDMAEMA$_{73}$ (P1) ($M_n = 11,400$, PDI = 1.08) and PDMAEMA$_{165}$ (P3) ($M_n = 26,200$, PDI = 1.04) macroCTAs were purified by precipitation into hexanes.
General Procedure for the RAFT Synthesis of \( P(\text{DMAEMA-}b\text{-NIPAM}) \)

NIPAM (0.294 g, 2.00 mmol), PDMAEMA\(_{73}\) (0.10 g), and V-501 (0.381 mg, 0.00137 mmol) were dissolved in 1 mL of dioxane were added to a 10 mL flask. After sparging with nitrogen for 30 min, the reaction was allowed to proceed at 80 \(^\circ\)C for 6 h (42 \% conversion). The reaction was then quenched by cooling the reaction vessel in an ice bath and exposure to air. The product \( P(\text{DMAEMA}_{73}-b\text{-NIPAM}_{99}) \) (\( P2 \)) (\( M_n = 22,900, \) PDI = 1.14) was purified by dialysis against deionized water and isolated by lyophilization.

Additionally, a series of block copolymers of DMAEMA and NIPAM was synthesized by the chain extension of PDEAEMA\(_{165}\). As an example, NIPAM (0.80 g, 7.07 mmol), PDMAEMA\(_{165}\) (0.80 g), and V-501 (1.63 mg, 0.0058 mmol) were dissolved in 4.8 mL of dioxane and added to a 10 mL round bottom flask. After sparging with nitrogen for 30 min, the reaction was allowed to proceed at 70 \(^\circ\)C for 3 h. The reaction mixture was then quenched by cooling the reaction vessel in an ice bath and subsequent exposure to air. The resultant block copolymers, \( P4 \) (\( M_n = 37,700, \) PDI = 1.10), \( P5 \) (\( M_n = 49,000, \) PDI = 1.17), and \( P6 \) (\( M_n = 75,400, \) PDI = 1.17), were purified by precipitation in hexanes (3x), dissolved in water, and isolated by lyophilization.
Scheme III-1. Preparation of multi-responsive block copolymers of DMAEMA and NIPAM via RAFT polymerization.

Figure III-2. PDMAEMA macroCTAs and block copolymers of NIPAM and DMAEMA synthesized by RAFT polymerization.

General Procedure for the RAFT Synthesis of PDEAEMA

A solution of CEP (71.0 mg, 0.270 mmol), DEAEMA (5.0 g, .027 mol), and V-501 (15.1 mg, 0.054 mmol) in 10 mL of deionized water was added to a 50 mL round bottom flask. Concentrated HCl was added to the solution to lower the pH to 4.5 to
ensure the PDEAEMA polymer remained soluble and to limit hydrolysis of the CTA. The solution was then sparged with nitrogen for approximately 30 min, and the flask was placed in a preheated oil bath at 70 °C. The reaction was terminated after 8 h by quenching the reaction tube in liquid nitrogen followed by exposure to air. The product was purified by dialysis against DI water (pH 4.5) for 3 days followed by lyophilization.

General Procedure for the RAFT Synthesis of P(DEAEMA-b-NIPAM)

The PDEAEMA<sub>98</sub>-CEP macroCTA was chain extended with NIPAM to yield two diblock copolymers following a similar procedure. For example, NIPAM (1.7 g, 10.6 mmol), PDMAEMA<sub>98</sub> (1.0 g), and VA-044 (17.6 mg, 0.054 mmol) were dissolved in 6 mL of DI water and added to a 25 mL round bottom flask. After sparging with nitrogen for 30 min, the reaction was allowed to proceed at 25 °C for 12 h. The reaction mixture was then quenched by cooling the reaction vessel in liquid nitrogen and exposure to air. The product was purified by dialysis against DI water (pH 4.5) for 3 days followed by lyophilization.

Self-Assembly of Block Copolymers

Self-Assembly of Block Copolymers of DMAEMA and NIPAM

Copolymers were dissolved at concentrations varying between 0.01 (0.1 mg/mL) and 0.1 wt% (1.0 mg/mL) directly in HPLC grade water containing 0, 50, or 200 mM NaCl. The pH was subsequently adjusted to 5, 7, or 9 using 0.1 N HCl or NaOH. Self-assembly of the block copolymers was then induced by increasing the temperature above the critical aggregation temperature (CAT) of the block copolymer.

Self-Assembly of Block Copolymers of DEAEMA and NIPAM

Copolymers were dissolved directly in deionized water at a concentration of 0.01 wt% (0.1 mg/mL). For temperature-induced assembly, the pH was adjusted to 5.0 using 0.1 N HCl or 0.1 N NaOH, and the temperature was slowly increased to 50 °C (1 °C/min).

Reversible Shell Cross-Linking of Self-Assembled Nanostructures

Shell Cross-Linking of P(DMAEMA-b-NIPAM) Nanostructures via AuNP Formation

The P(DMAEMA-b-NIPAM) solutions of varying copolymer concentration (0.01, 0.05, 0.1 wt%), pH (5.0, 7.0, and 9.0), and salt concentration (0, 50, and 200 mM NaCl) were heated to 50 °C (1.0 °C/min) to induce self-assembly. After 30 min, 2 to 5 µL of a preheated solution of sodium tetrachloroaurate (III) dihydrate solution (NaAuCl₄) at pH 6.5 was added to the copolymer solution at 50 °C to give a DMAEMA to Au ratio of 10 to 1. The mixed solution was allowed to stir at 50 °C for 48 hours prior to being cooled to room temperature for analysis.

General Procedure for the Ligand Exchange Reaction to Reverse AuNP Cross-Linking

In order to reverse AuNP-“locking”, 1 mL of a solution containing AuNP cross-linked micelles or vesicles was reacted with an appropriate volume of 1 mM cysteamine
or 1 mM PEG-SH to yield a thiol to DMAEMA ratio of 10. The mixture was allowed to stir for 48 h prior to centrifugation at 13,000 rpm for 1 hr to remove liberated polymer from the thiol-stabilized AuNPs. After removal of the supernatant, the AuNPs were redispersed in 1.0 mL of HPLC grade water for analysis.

(Co)Polymer Characterization

Size Exclusion Chromatography

SEC was used to determine the number-average molecular weight ($M_n$) and polydispersity indices (PDIs) for all homo- and block copolymers. The PDEAEMA and PDEAEMA macroCTAs were analyzed by aqueous size exclusion chromatography (ASEC) using an aqueous eluent of 1.0 wt% acetic acid/0.1 M Na$_2$SO$_4$. A flow rate of 0.25 mL/min, Eprogen Inc. columns [CATSEC1000 (7μ, 50×4.6), CATSEC100 (5μ, 250×4.6), CATSEC1000 (7μ, 250×4.6) and CATSEC300 (5μ, 250×4.6)], a Wyatt Dawn EOS multiangle laser light scattering detector (λ = 690 nm), and an Optilab DSP interferometric refractometer (λ = 690 nm) were used. Wyatt DNDC for Windows was used for the macroCTA $dn/dc$ determination. The homo- and block copolymers were analyzed using a DMF eluent (0.02 M LiBr) at a flowrate of 1.0 mL/min in combination with Viscotek I-Series Mixed Bed low-MW and mid-MW columns, and a Viscotek-TDA 302 (RI, viscosity, 7 mW 90° and 7° true low angle light scattering detectors (670 nm)) at 35 °C. The $dn/dc$ of each (co)polymer was determined in DMF at 35 °C using a Viscotek refractometer and Omnisec software.
Copolymer Characterization using $^1$H NMR Spectroscopy

$^1$H NMR measurements were performed with a temperature-controlled Varian UNITY INOVA spectrometer operating at a frequency of 499.8 MHz. P(DMAEMA-$b$-NIPAM) samples were prepared in D$_2$O (HOD internal standard) and spectra were attained for each copolymer at 5 °C increments from 25 to 50 °C. P(DEAEMA-$b$-NIPAM) samples were prepared in D$_2$O (HOD internal standard), and spectra were recorded for each copolymer at temperatures of 25 and 50 °C and pH values of 5.0 and 9.0. Block copolymer compositions were determined by comparing resonances associated with the two blocks in the spectra recorded at 25 °C.

Characterization of Self-Assembled Nanostructures

Dynamic and Static Light Scattering

Dynamic light scattering (DLS) studies investigating the effect of incremental temperature and pH changes were conducted using a Malvern Instruments Zetasizer Nano series instrument equipped with a 4 mW He-Ne laser operating at $\lambda = 632.8$ nm, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple tau digital correlator electronics system. Dispersion Technology Software 5.03 (Malvern Instruments) was used to record and analyze the data to determine particle size distributions.

Variable-angle DLS and static light scattering (SLS) measurements were made using incident light at 633 nm from a Spectra Physics HeNe operating at 40 mW. The angular dependence of the autocorrelation functions was measured using a Brookhaven Instruments BI-200SM goniometer with an avalanche photodiode detector and TurboCorr correlator. Correlation functions were analyzed according to the method of cumulants.
using the companion software. All data reported correspond to the average decay rate obtained from the second cumulant fit. Apparent diffusion coefficients ($D_{\text{app}}$) were obtained from the slope of the relaxation frequency ($\Gamma$) versus $q^2$ where

$$q = \frac{4\pi n}{\lambda} \sin \left(\frac{\theta}{2}\right),$$

(6)

\(\lambda\) is the wavelength of the incident laser (633 nm), \(\theta\) is the scattering angle, and \(n\) is the refractive index of the media. The hydrodynamic radius ($R_h$) was then calculated from the Stokes-Einstein equation (Equation 7)

$$R_h = \frac{k_B T}{6\pi \eta D_{\text{app}}}$$

(7)

where \(k_B\) is the Boltzmann constant, \(T\) is the temperature, and \(\eta\) is the viscosity of the medium.

Angular–dependent SLS experiments were performed on aqueous polymer solutions with the same instrument as described above. The radius of gyration ($R_g$) of the assemblies was determined from the angular dependence of the scattering intensity. A Zimm plot of the scattering intensity ($I_{\text{ex}}$) versus the square of the scattering vector ($q$) was used to determine the $R_g$. A Berry plot ($I_{\text{ex}}^{-1/2}$ vs. $q^2$) is used in instances where a Zimm treatment results in upward curvature of the data due when $qR_g \geq 1$.

Solutions were prepared by dissolving the polymer into purified water to a concentration of 0.01 wt%. Samples were agitated to ensure complete dissolution and then filtered through a 0.45 \(\mu\)m PVDF syringe-driven filter (Millipore) directly into the scattering cell. Samples were then sonicated and allowed to reach thermal equilibrium prior to measurements.
**Zeta Potential Measurements**

Zeta potential measurements were performed on an aqueous 0.01 wt% copolymer solution using a Malvern Instruments Zetasizer Nano series instrument using the Smoluchowsky relationship. The solution pH was adjusted by the addition of 0.1 M HCl or 0.1 M NaOH.

**Transmission Electron Microscopy**

Transmission electron microscopy measurements were conducted using a JEOL JEM-2100 electron microscope at an accelerating voltage of 200 kV. The specimens were prepared by placing a 5 µL drop of the nanostructure solution on a carbon-coated copper grid followed by water evaporation at either 25 or 50 °C. The grids were subsequently stained using a 1 wt% phosphotungstic acid solution which stained the amino functionality of DEAEMA.\(^{220}\)
CHAPTER IV
RESULTS AND DISCUSSION

This work may be divided into four sections. The first section concerns work performed in collaboration with Dr. Yuting Li on the self-assembly of vesicles (polymersomes) from a block copolymer of 2-(dimethylamino)ethyl methacrylate (DMAEMA) (M17) and N-isopropylacrylamide (NIPAM) (M1) and their subsequent “locking” through the in situ gold nanoparticle (AuNP) formation. The second section details work performed on a series of DMAEMA and NIPAM block copolymers to investigate the effect of various experimental parameters (copolymer composition and concentration, solution pH value, and ionic strength) on the temperature-induced aggregation behavior and the resultant solution morphology. The formed nanostructures were subsequently cross-linked with AuNPs and investigated using dynamic light scattering (DLS) and transmission electron microscopy (TEM). The ability to reverse the cross-linking of the AuNP-“locked” nanostructures through the use of ligand exchange reactions is discussed in the third section. The fourth section concerns the investigation of copolymer composition of two dually-responsive block copolymers of 2-(diethylamino)ethyl methacrylate (DEAEMA) (M19) and NIPAM capable of “schizophrenic” aggregation into spherical micelles and vesicles.
Section I. *In Situ* Formation of Gold-“Decorated” Vesicles from a RAFT-synthesized, Thermally Responsive Block Copolymer

*Overview*

The delivery of drugs from nanostructured assemblies derived from block copolymers has been extensively studied in recent years. However, despite the recognized potential as drug delivery vehicles, self-assembling structures are inherently limited due to multimer dissociation upon injection into the bloodstream. These amphiphilic aggregates experience a large dilution effect which leads to concentrations below the critical aggregation concentration and eventually burst release of the drug payload.\(^{221}\) This can be avoided by cross-linking the nanostructure. Unfortunately, the core cross-linking often decreases drug carrying capacity and thus hinders application as a drug delivery vehicle.\(^{222}\) An alternative approach is to cross-link the shell of the self-assembled aggregate. Previous work in the McCormick research group has focused on the synthesis of thermally- and pH-responsive block copolymers, their self-assembly behavior into micelles and vesicles in aqueous solution, and their subsequent reversible shell cross-linking using cleavable disulfide bonds\(^{223, \ 224}\) or salt-\(^{225-228}\) and pH-reversible\(^{228}\) interpolyelectrolyte complexes.

AuNPs have been the focus of intense research over the past decade due to their unique properties and potential application in many areas including biomedical materials, optics, and electronics.\(^{229}\) Thiol chemistry has widely been used to modify the surface of AuNPs with synthetic polymers\(^{212, \ 217, \ 230, \ 231}\) and biomacromolecules.\(^{232-234}\) Lowe and coworkers working in the McCormick Research Group reported the NaBH\(_4\) reduction of dithioester-terminated, water-soluble polymers directly in water in the presence of noble
metal salts including NaAuCl₄ to yield sterically and electrostatically stabilized zero-valent metal nanoparticles.²¹² Recently, there have been numerous reports utilizing amine-containing copolymers that can act as both a reducing agent and a stabilizing agent.²¹³-²¹⁷

Building on the previous experience in the McCormick Research Group, herein we report a thermally-responsive vesicle system that is easily decorated with gold nanoparticles. These vesicles are formed by the self-assembly of the thermally-responsive P(DMAEMA-b-NIPAM) (P2) in aqueous solution. By simply mixing the polymer solution with a NaAuCl₄ solution at 50 °C under specified conditions, AuNP-containing vesicles can be obtained. This procedure (outlined in Scheme IV-1) does not require the addition of an external reducing agent and results in stabilized vesicles which remain dispersed in aqueous solution upon cooling to room temperature.

**Scheme IV-1.** Formation of thermally responsive vesicles self-assembled from P(DMAEMA₇₃-b-NIPAM₉₉) decorated with AuNPs.
RAFT Synthesis of P(DMAEMA$_{73}$-b-NIPAM$_{99}$)

Reversible addition-fragmentation chain-transfer (RAFT) polymerization was utilized in the synthesis of the diblock copolymer composed of pH-responsive DMAEMA (M$_{17}$) and the thermally responsive NIPAM (M$_{1}$) segments. In order to design diblocks with low polydispersity indices (PDIs) and compositions for a) vesicle formation above the lower critical solution temperature (LCST) and b) maintenance of electrosteric stabilization of the resulting gold-decorated vesicles, it was necessary to optimize reaction conditions, monomer concentration, and blocking order. It was determined the DMAEMA should be polymerized first using 4-cyanopentanoic acid dithiobenzoate (CTP) (CTA$_{1}$) as the RAFT chain transfer agent (CTA). The resulting macroCTA could then be utilized for efficient polymerization of NIPAM. Considering these aforementioned design criteria, we first synthesized the PDMAEMA macroCTA (P$_{1}$), stopping conversion at 69% to maintain end group fidelity and molecular weight control; the number average molecular weight ($M_{n}$) and PDI were determined to be 11,400 and 1.08, respectively. This macroCTA was then chain extended with NIPAM yielding a well defined diblock copolymer, P(DMAEMA$_{73}$-b-NIPAM$_{99}$) (P$_{2}$), with $M_{n}$ and PDI values of 22,900 and 1.14, respectively.
Scheme IV-2. Preparation of multi-responsive block copolymers of DMAEMA and NIPAM via RAFT polymerization.

Figure IV-1. DMF SEC traces for (a) PDMAEMA\textsubscript{73} macroCTA and (b) P(DMAEMA\textsubscript{73}-\textit{b}-NIPAM\textsubscript{99}).

Self-Assembly and AuNP-“Locking” of P(DMAEMA\textsubscript{73}-\textit{b}-NIPAM\textsubscript{99}) Vesicles

A 0.01 wt% solution of this diblock copolymer at pH 7.4 was then prepared and the aggregation behavior studied as a function of temperature (Figure IV-2) utilizing DLS. A sharp transition at 38 °C is observed from unimers with hydrodynamic diameter ($D_h$) below 8 nm to vesicles with average $D_h$ of 140 nm. This process is completely
reversible. Also shown are the $^1$H NMR spectra (Figure IV-3) of the homo and block copolymers at selected temperatures. At 25 °C, the diblock copolymer is fully solvated, and signals associated with each block are observed. An increase in the solution temperature to 50 °C causes the NIPAM signal to become broadened and significantly suppressed while the DMAEMA signal remains, for the most part, unattenuated. This, in addition to zeta potential measurements (Figure IV-4), reflects the presence of the positively-charged PDMAEMA blocks located on the surface of the particles at pH 7.

![Figure IV-2](image)

**Figure IV-2.** Variation of hydrodynamic diameter with temperature for the P(DMAEMA$_{73}$-$b$-NIPAM$_{99}$) at 0.01 wt % in aqueous solution at pH 7.4.
Figure IV-3. The $^1$H NMR spectra of the homo and block copolymers in D$_2$O at selected temperatures. (A) PDMAEMA$_{73}$, 25 °C, (B) P(DMAEMA$_{73}$-b-NIPAM$_{99}$), 25 °C, and (C) 0.01 wt % P(DMAEMA$_{73}$-b-NIPAM$_{99}$), 50 °C.

Figure IV-4. Zeta potential vs. pH curves obtained for the vesicles self-assembled from P(DMAEMA$_{73}$-b-NIPAM$_{99}$).

Recently, Armes et al. showed that polymers containing DMAEMA functionality can be utilized to reduce AuCl$_4^-$ counterions to zerovalent gold, and, at the same time, stabilize the resulting gold nanoparticles.$^{217}$ In our experiments, after dissolving P(DMAEMA$_{73}$-b-NIPAM$_{99}$) (P2) at 0.01 wt%, we first allowed vesicle formation to occur at 50 °C. The resulting solution was then mixed with the NaAuCl$_4$ solution in a
10:1 molar ratio of DMAEMA units:NaAuCl₄. The pH of the initial polymer solution was 7.4, reaching an equilibrated pH value of 6.4 after the addition of NaAuCl₄. DLS analysis (Figure IV-5) shows that vesicle size and size distribution increased slightly with this reaction (Figure IV-5, curve b to curve c), which is attributed to increased protonation of the PDMAEMA segments during equilibration and gold complex reduction. The mixed solution was kept at 50 °C for 2 days, after which time the solution temperature was lowered to 25 °C. DLS analysis detected no dissociation into unimers. It appears that the vesicle structure is “fixed” since the thermally responsive vesicles do not dissociate into unimers at 25 °C. The vesicle size increased at 25 °C (Figure IV-5, curve d) relative to that at 50 °C (Figure IV-5, curve c) due to the swelling behavior of the vesicles as the PNIPAM block becomes more hydrophilic at 25 °C. It should be noted that the molar ratio of the PDMAEMA and NaAuCl₄ is critical for the formation of the gold nanoparticles-decorated vesicles. In our experiment, as previously mentioned, PDMAEMA:NaAuCl₄ was kept at 10:1. When the ratio was lowered to 5:1, the decrease in the hydrophilicity of the PDMAEMA/NaAuCl₄ block results in precipitation as manifested by the onset of turbidity. Compared to chemical cross-linking of vesicles, this method is quite attractive since it allows for simultaneous gold nanoparticle formation, “locking” of the resulting structure, and still permits long-term stability in aqueous media.
Figure IV-5. Dynamic light scattering size distribution of a 0.01 wt % P(DMAEMA$_{73}$-b-NIPAM$_{99}$) solution: a) 25 °C; b) 50 °C; c) 50 °C after *in situ* reduction of NaAuCl$_4$; d) after *in situ* reduction of NaAuCl$_4$ upon lowering temperature to 25 °C.

Shown in Figure IV-6a is a TEM of gold-decorated structures. The structures are spherical and possess morphology consistent with that of vesicles. The bound gold nanoparticles function to “stain” these structures, enhancing the TEM image. The formation of the gold nanoparticles decorating the vesicles is also confirmed by a gradual change to red after mixing the polymer solution with the NaAuCl$_4$ solution. Figure IV-6b shows the UV-vis absorbance spectrum that indicates a maximum absorbance at 525 nm, which corresponds to reported surface plasmon resonance of gold nanoparticles.
In order to demonstrate that the morphology observed in Figure IV-6a could not be attributed to association induced by mere reduction of AuCl$_4^-$ counterion associated with the protonated PDMAEMA segments, a control experiment was conducted under identical reaction conditions and block copolymer concentration; however the temperature was maintained at 25 °C – well below the experimentally determined LCST. Within 48 hours, the solution turned the characteristic red color, indicating successful reduction; however, the stabilized gold nanoparticles, roughly 20 nm in diameter, had no resemblance to the vesicles formed at 50 °C (Figure IV-7).
Figure IV-7. Transmission electron micrograph of the control experiment P(DMAEMA$_{73}$-$b$-NIPAM$_{99}$) stabilized gold nanoparticles formed at 25 °C.
Overview

Self-assembly of block copolymers with precisely defined structures is the subject of intensive research for applications in nanomedicine. Micelles formed from amphiphilic block copolymers in aqueous solution, for example, have been investigated in recent years as potential carriers for therapeutic and diagnostic agents.\(^{235}\) Micelles are not, however, the only structures formed from self-assembling amphiphilic block copolymers, rather they are part of a morphological continuum that includes worm-like micelles and polymeric vesicles (commonly referred to as polymersomes in comparison to the liposomes).\(^{158-160}\) By controlling the packing of polymer chains, specific morphologies from self-assembled amphiphilic block copolymers can be obtained. The organization of the polymer chains, which is influenced by a number of factors including molecular weight, polymer composition, polydispersity, and chain architecture, can be described by the packing parameter, \(p\), which is defined as:

\[
p = \frac{v}{a_h l_c}
\]  

(8)

where \(v\) is the volume of the hydrophobic chains, \(a_h\) is the optimal head group area, and \(l_c\) is the length of the hydrophobic tail.\(^{161}\) The value of \(p\) is often used to predict which morphology is favored (spherical micelles when \(p \leq 1/3\), cylindrical micelles when \(1/3 \leq p \leq 1/2\), and vesicles (polymersomes) when \(1/2 \leq p \leq 1\)).\(^{162}\) More recently, Discher and Eisenberg developed an empirical relationship between the block copolymer composition and the self-assembled morphologies.\(^{163}\) Spherical micelles are expected for polymers
with hydrophilic mass fractions \((f)\) greater than 45 \%, while copolymers with \(f \approx 35 \pm 10 \%\) typically assemble into polymersomes.

Stimuli-responsive block copolymers afford a facile method for tuning the hydrophilic mass fraction to provide access to various solution morphologies. Such “smart” materials exhibit dramatic changes in properties in response to the alteration of external stimuli, such as temperature, pH, and ionic strength.\(^{226, 236-240}\) A number of investigations have documented reversible switching between morphologies by tuning the hydrophilic to hydrophobic ratio with changes in pH\(^{241-244}\) and temperature.\(^{245-247}\) Systems responsive to two stimuli provide an even greater level of control and are of immense importance for biologically relevant applications.\(^{248}\) Certain homopolymers also display such dual responsiveness. For example, PDMAEMA is both thermo- and pH-responsive.\(^{249-251}\)

Herein, we report the synthesis of a series of block copolymers of DMAEMA (M\(^{17}\)) and NIPAM (M\(^{1}\)) utilizing RAFT polymerization. RAFT provides a facile method of preparing the desired block copolymer architecture while maintaining precise control over the macromolecular characteristics (molecular weight, copolymer composition, functionality, etc.) that dictate nanostructure morphology.\(^{252-255}\) The block lengths were varied to give hydrophilic mass fractions necessary for the formation of micelles, worm-like micelles, and vesicles above the LCST.\(^{164}\) Additionally, polymer concentration, pH, and ionic strength have been varied to determine their respective effects on the resulting assembled morphology. The nanostructures were subsequently cross-linked by the \textit{in situ} reduction of NaAuCl\(_4\) to AuNPs within the PDMAEMA layer as discussed in the previous section.
**RAFT Synthesis of Multiply-Responsive P(DMAEMA-b-NIPAM)**

Diblock copolymers of DMAEMA (M<sub>17</sub>) and NIPAM (M<sub>1</sub>) were synthesized according to Scheme III-1. DMAEMA was first polymerized using CTP (CTA<sub>1</sub>) and V-501 (I<sub>3</sub>) in dioxane to produce a PDMAEMA macroCTA. Monomer conversion was kept below 70% in order to maintain the dithioester chain-end functionality for efficient polymerization of the subsequent PNIPAM block. The PDMAEMA<sub>165</sub> macroCTA had M<sub>n</sub> and PDI values of 26,200 g/mol and 1.04, respectively. The PDMAEMA<sub>165</sub> macroCTA was chain extended with NIPAM to give three block copolymers with degrees of polymerization of 102 (P<sub>4</sub>), 202 (P<sub>5</sub>), and 435 (P<sub>6</sub>). SEC chromatograms of the copolymer series are shown in Figure IV-8. All of the SEC traces are unimodal and the PDIs are low (< 1.2) indicating near-quantitative blocking efficiency and controlled polymerization. Low molecular weight tailing of the SEC chromatograms is due to the interaction of the PDMAEMA block with the GPC columns used for the analysis of the block copolymer system. Analysis of the PDMAEMA macroCTA via ASEC utilizing CATSEC columns specifically tailored for cationic polymers (but not appropriate for PNIPAM) shows a narrow peak with no perceptible tailing at higher elution volumes (Figure IV-9). The molecular weight and composition data of the diblock copolymer series are summarized in Table IV-1.
Scheme IV-3. Preparation of multi-responsive block copolymers of DMAEMA and NIPAM via RAFT polymerization.

Figure IV-8. SEC chromatograms for the chain extension of PDMAEMA$_{165}$ macroCTA to yield three DMAEMA and NIPAM block copolymers using RAFT polymerization.
Table IV-1. Summary of DMAEMA and NIPAM block copolymer series molecular weight and composition.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n^a$</th>
<th>PDI$^a$</th>
<th>Hydrophilic Mass Fraction (%) at 50 °C, pH 5$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMAEMA$_{165}$ (P3)</td>
<td>26,200</td>
<td>1.04</td>
<td>100</td>
</tr>
<tr>
<td>P(DMAEMA$<em>{165}$-b-NIPAM$</em>{102}$) (P4)</td>
<td>37,700</td>
<td>1.10</td>
<td>70</td>
</tr>
<tr>
<td>P(DMAEMA$<em>{165}$-b-NIPAM$</em>{202}$) (P5)</td>
<td>49,000</td>
<td>1.17</td>
<td>53</td>
</tr>
<tr>
<td>P(DMAEMA$<em>{165}$-b-NIPAM$</em>{435}$) (P6)</td>
<td>75,400</td>
<td>1.17</td>
<td>35</td>
</tr>
</tbody>
</table>

$^a$As determined by SEC. $^b$Determined by $^1$H NMR in D$_2$O.

**Temperature-Induced Assembly of P(DMAEMA$_{165}$-b-NIPAM$_y$)**

**Effect of Block Copolymer Composition.** RAFT provides a facile technique for preparing a well-defined series of amphiphilic diblock copolymers of preselected compositions which can be utilized to assess the importance of block lengths on the temperature-responsive assembly. Specific copolymer compositions were targeted to produce hydrophilic mass fractions corresponding to spherical micelles, worm-like
micelles, and vesicles as predicted by Discher and Eisenberg for amphiphilic block copolymers with a permanently hydrophobic block.\textsuperscript{163,164}

Examining the temperature-responsive self-assembly at 0.01 wt\% and a pH of 5.0 for the three block copolymers utilizing DLS reveals a strong dependence of the aggregation behavior on the length of the PNIPAM block. As shown in Figure IV-10, the copolymer with the shortest NIPAM block, P(DMAEMA\textsubscript{165}-b-NIPAM\textsubscript{102}) (P\textsubscript{4}), does not display a critical aggregation temperature (CAT) and remains dispersed as unimers over the temperature range studied. Increasing the degree of polymerization (DP) of the hydrophobic block from 102 to 202 (P\textsubscript{5}) results in the onset of aggregation at 38 °C and aggregates of hydrodynamic diameter values of \( \approx 220 \) nm above 44 °C. Further increasing the DP of the PNIPAM block to 435 (P\textsubscript{63}) lowers the CAT to 36 °C while maintaining aggregate sizes of 210 nm above 36 °C. The DLS results are summarized in Table IV-2.

![Figure IV-10](image-url)

**Figure IV-10.** Hydrodynamic diameter vs. temperature data for the three DMAEMA and NIPAM block copolymers showing the effect of block copolymer composition on the self-assembly behavior in aqueous solution (0.01 % (w/w) concentration, pH 5.0).
<table>
<thead>
<tr>
<th>NaCl Concentration (mM)</th>
<th>Solution pH</th>
<th>0 mM</th>
<th>50 mM</th>
<th>200 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>DMAEMA₁₆₅-b-NIPAM₁₀₂</td>
<td>13.3 ± 4.3</td>
<td>246 ± 7.8</td>
<td>570 ± 10.2</td>
<td>13.3 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>232 ± 6.6</td>
<td>276 ± 5.3 (66 %)</td>
<td>643 ± 14.6</td>
<td>94.1 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>210 ± 0.6</td>
<td>179 ± 1.6</td>
<td>3250 ± 89.5</td>
<td>108 ± 1.2</td>
</tr>
<tr>
<td>DMAEMA₁₆₅-b-NIPAM₃₁₅</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table IV-2. Hydrodynamic diameters measured from DLS for block copolymer solutions (0.01 wt%) at 50 °C under varying pH and NaCl concentration.
**Effect of block copolymer concentration.** The size of the aggregates is also strongly influenced by the copolymer concentration. Figure IV-11 shows the effect of increasing concentration of P(DMAEMA$_{165}$-b-NIPAM$_{102}$) (P4) on the unimer-to-nanostructure transition at pH 5.0. As discussed above, at a copolymer concentration of 0.01 wt% (0.1 mg/mL), the copolymer does not undergo a thermally-induced transition from unimers to macromolecular aggregates. This can be attributed to the concentration being below the critical aggregation concentration (CAC). Increasing the copolymer concentration to 0.05 wt% (0.5 mg/mL) leads to the onset of aggregation at 44 °C and 480 nm nanostructures above 50 °C. Above the CAC, the size of the polymeric aggregates and the CAT show a marked dependence on the copolymer concentration. Further increasing the concentration to 0.1 wt% (1.0 mg/mL) decreases the onset of aggregation to 42 °C and leads to aggregates of 580 nm above 46 °C.

![Figure IV-11. Effect of block copolymer concentration on the temperature-responsive aggregation of P(DMAEMA$_{165}$-b-NIPAM$_{102}$) in aqueous solution (pH 5.0).](image-url)
Effect of solution pH value. The DMAEMA repeat units that comprise the hydrophilic stabilizing blocks are tertiary amines which can be reversibly protonated by adjusting the pH of the solution. Due to the complex stimuli-responsive behavior of the PDMAEMA stabilizing block, altering the pH should have a dramatic effect on the solution aggregation behavior of the block copolymers. Previous studies have reported a strong effect by chain ends and comonomers on the LCST of PNIPAM.\textsuperscript{57,256} As the pH of the copolymer solution is increased, the hydrophilicity of the PDMAEMA stabilizing block decreases. At pH 5, the DMAEMA moieties (pKa 7.3) are 99 \% protonated which increases the hydrophilicity of the block copolymer so that P(DMAEMA\textsubscript{165}-b-NIPAM\textsubscript{102}) (P\textsubscript{4}) does not possess sufficient hydrophobic character for aggregation in the temperature range of this study at a concentration of 0.01 wt\% (Figure IV-12a). At pH 7, the DMAEMA units are \sim{} 65 \% ionized, leading to copolymer aggregates with hydrodynamic diameters of 246 nm above 50 °C. Further increasing the pH to 9.0 decreases the ionization of the PDMAEMA to approximately 2 \%. This greatly decreases the hydrophilicity of the copolymer system lowering the CAT to 38 °C. Additionally, due to the deprotonation of most of the DMAEMA units, the PDMAEMA block becomes temperature-responsive in the range of this study. At this pH, the aggregates increase in size to 570 nm.
Figure IV-12. Variation of hydrodynamic diameter with temperature of (a) P(DMAEMA<sub>165-b-NIPAM</sub><sub>102</sub>) and (b) P(DMAEMA<sub>165-b-NIPAM</sub><sub>202</sub>) in aqueous solutions (0.01 % (w/w)) of varying pH.

Interestingly, this behavior is not observed for P(DMAEMA<sub>165-b-NIPAM</sub><sub>202</sub>) (P5). Figure IV-12b shows the effect of pH on the temperature-induced aggregation of 0.01 wt% solutions. At pH values of 5.0 and 9.0, the aggregation behavior of P(DMAEMA<sub>165-b-NIPAM</sub><sub>202</sub>) follows the expected trends discussed above. At pH 7.0,
however, two distinct populations arise above 46 °C. One population has a size slightly larger than the aggregates formed at pH 5.0 (~ 240 nm). The smaller-sized population has a hydrodynamic diameter of approximately 60 nm. This mixed population system will be discussed in further detail in a subsequent section.

**Effect of electrolyte concentration.** Since the PDMAEMA block is a polyelectrolyte, the addition of salt should screen the cationic charges along the polymeric backbone, decreasing the rigidity and subsequently affecting the packing behavior in polymeric aggregates above the CAT of the DMAEMA and NIPAM block copolymers. The addition of NaCl should also have a “salting out” effect on the NIPAM block that would lower the CAT value. Figure IV-13 shows the effect that addition of salt has on the aggregates formed from P(DMAEMA$_{165}$-b-NIPAM$_{102}$) (P4) at a concentration of 0.01 wt% and a solution pH of 7.0. As discussed earlier, in the absence of salt, the block copolymer forms aggregates of 232 nm. When the copolymer is dissolved in an aqueous 50 mM NaCl solution, the size of the aggregates decreases to 94 nm, but the CAT is unchanged. Increasing the NaCl concentration to 200 mM leads to a further decrease in the aggregate size to 60 nm and also decreases the CAT by 4 °C.
Figure IV-13. Variation of hydrodynamic diameter with temperature of DMAEMA$_{165}$-$b$-NIPAM$_{102}$ in aqueous solution (0.01 wt%, pH 7.0) at varying NaCl concentrations.

AuNP Cross-Linking of Assembled Nanostructures

The cross-linking of the self-assembled nanostructures by the in situ formation of AuNPs was accomplished using a procedure modified from our previous work according to Scheme IV-4. The block copolymer solutions were heated to 50 °C at a rate of 1 °C/min and allowed to stir for 30 min prior to the addition of the NaAuCl$_4$. A small volume (2-5 µL) of a NaAuCl$_4$ solution was then added to give a DMAEMA:Au ratio of 10:1. A higher ratio results in incomplete cross-linking while a lower ratio leads to precipitation of the AuNPs in some samples. The AuNP cross-linking provides a facile method to “lock” the self-assembled nanostructures and further investigate the morphologies of the aggregates studied by DLS. The cross-linked structures are easily analyzed by TEM since the AuNPs act as a staining agent for the nanostructures.
**Scheme IV-4.** Idealized formation of gold cross-linked nanostructures formed from the temperature-induced self-assembly of DMAEMA and NIPAM block copolymers.

Figure IV-14 shows the size distribution determined from DLS and the corresponding TEM images of the AuNP cross-linked aggregates formed from a solution of P(DMAEMA$_{165}$-b-NIPAM$_{102}$) (P4) dissolved in 200 mM NaCl at a pH of 7.0. Prior to cross-linking, the aggregates possessed average hydrodynamic diameters of 58 nm at 50 °C. After the cross-linking reaction, the solution temperature was lowered below the CAT to ambient temperature and the aggregate sizes increased to 72 nm. This increase in size is attributed to the rehydration of the PNIPAM core. In the TEM micrographs spherical particles ranging from 30 nm to 80 nm are observed. From the sizes determined by DLS and TEM, one can conclude that under these conditions P(DMAEMA$_{165}$-b-NIPAM$_{102}$) polymers form a simple core-shell micelle morphology as predicted based on the hydrophilic mass fraction ($f$=68 %) of this copolymer.$^{164}$
As discussed earlier, when a pH 7.0 solution of P(DMAEMA$_{165}$-b-NIPAM$_{102}$) (P5) is heated to 50 °C, Contin analysis of DLS data reveals two distinct populations. Before cross-linking, two distributions appear at 61 and 237 nm. After cross-linking, both shift to larger hydrodynamic diameters, 78 and 289 nm, respectively. TEM (Figure IV-15) provides additional evidence for two populations. The smaller sized distribution arises from spherical micelles while the larger size is attributed to the worm-like structures. These elongated structures have lengths approaching 500 nm with diameters ranging from 50 nm to 100 nm. The presence of two coexisting morphologies is not surprising and has been reported previously.$^{164}$ Significantly, the hydrophilic mass fraction of this system (48 wt%) corresponds to the numbers proposed for the formation of worm-like structures.$^{164}$

**Figure IV-14.** (a) Dynamic light scattering of micelles formed from aqueous solution (0.01 wt%, pH 7.0, 200 mM NaCl) of P(DMAEMA$_{165}$-b-NIPAM$_{102}$) before and after cross-linking. (b) TEM micrograph of AuNP cross-linked P(DMAEMA$_{165}$-b-NIPAM$_{102}$) micelles.
Figure IV-15. (a) Dynamic light scattering of spherical and worm-like micelles formed from aqueous solution (0.01 wt%, pH 7.0) of P(DMAEMA\textsubscript{165}-\textit{b}-NIPAM\textsubscript{202}) before and after cross-linking. (b) TEM micrograph of AuNP cross-linked P(DMAEMA\textsubscript{165}-\textit{b}-NIPAM\textsubscript{202}) spherical and worm-like micelles.

When a 0.01 wt% aqueous solution (pH 7.0) of P(DMAEMA\textsubscript{165}-\textit{b}-NIPAM\textsubscript{435}) (P\textsubscript{6}) is heated to 50 °C, nanostructures of 179 nm are formed as determined from DLS. After \textit{in situ} gold nanoparticle formation to crosslink the aggregates, the apparent hydrodynamic diameters increase to 210 nm when the solution is cooled to room temperature (Figure IV-16). The electron micrograph of the cross-linked nanostructures shows aggregates with a vesicular morphology consistent with that reported in our previous work.\textsuperscript{75} Additionally, elongated vesicular structures are observed.
Figure IV-16. (a) Dynamic light scattering of vesicles formed from aqueous solution (0.01 wt%, pH 7.0) of DMAEMA$_{165}$-b-NIPAM$_{435}$ before and after cross-linking. (b) TEM micrograph of AuNP cross-linked DMAEMA$_{165}$-b-NIPAM$_{435}$ vesicles.
Section III. Reversible AuNP Shell Cross-linking of Nanostructures Derived from Stimuli-Responsive Diblock Copolymers

Overview

In the preceding sections, the synthesis, self-assembly, and AuNP cross-linking of multiply-responsive block copolymers of DMAEMA and NIPAM have been discussed. These AuNP-decorated systems are of interest in combining therapeutic delivery inherent to block copolymers and vesicles and potential diagnostic imaging in a “theranostic” vehicle for potential applications in nanomedicine. However, one disadvantage of using such shell cross-linked aggregates for drug delivery is that their large size prevents renal excretion. One method to circumvent both the dilution-effect and potential buildup of the aggregates in the kidneys is to use reversible cross-linking chemistries which allow the gradual breakdown of the cross-links after successful delivery. Recently, a major emphasis in our research has been the construction of stimuli-reversible cross-linked systems with cleavable disulfide bonds or salt- and pH-reversible interpolyelectrolyte complexes. Herein we describe the use of ligand exchange reactions in which the thiols cysteamine and PEG-SH are utilized to reverse the AuNP cross-linking of micelles and vesicles self-assembled in aqueous solution from P(DMAEMA\textsubscript{165}-b-NIPAM\textsubscript{102}) (P4) and P(DMAEMA\textsubscript{165}-b-NIPAM\textsubscript{435}) (P6), respectively.

Preparation and Shell Cross-Linking of Polymersomes

Utilizing the methods discussed in the previous section, the vesicle-forming P(DMAEMA\textsubscript{165}-b-NIPAM\textsubscript{435}) (P6) was self-assembled and cross-linked with AuNPs at 50 °C. Figure IV-17A shows the distribution of assembly sizes for unimers, vesicles and
“locked” vesicles measured at a fixed angle of 173° using a Malvern Instruments Zetasizer Nano light scattering instrument. The aqueous solution of P(DMAEMA_{165-b-NIPAM_{435}}) (0.01 wt%, pH 7.0) when heated to 50 °C (1 °C/min) self-assembles into aggregates having a hydrodynamic diameter (D_h) of 178 nm (Figure IV-17A, a). The *in situ* reduction of AuCl_4^- at 50 °C results in Au^{(0)} nanoparticles bound to the DEAEMA block, presumably through counterion exchange and subsequent chelation. The cross-linked nanostructures remain intact upon lowering the temperature to 25 °C. A slight increase in the hydrodynamic size (Figure IV-17A, b) is observed from the increased hydrophilicity of the PNIPAM segment.

**Scheme IV-5.** Reversible AuNP-“locking” of P(DMAEMA_{165-b-NIPAM_{435}}) vesicles accomplished by a ligand exchange of PDMAEMA for a thiolated stabilizing agent.
Figure IV-17. (A) DLS measurements showing the reversibility of the AuNP-“locking” of vesicles formed from P(DMAEMA$_{165}$-$b$-NIPAM$_{435}$). (a) 0.01 wt% P(DMAEMA$_{165}$-$b$-NIPAM$_{435}$) (pH 7.0, T = 50 °C), (b) AuNP cross-linked vesicles (T = 25 °C), AuNPs after ligand exchange with (c) cysteamine and (d) PEG-SH. (B) Angular dependent DLS and (C) SLS measurements for the AuNP cross-linked vesicles.

To provide greater insight into the morphology of the cross-linked structure, DLS and static light scattering (SLS) studies were performed at multiple angles using a Brookhaven BI-200SM goniometer with a TurboCorr correlator. A plot of the diffusion coefficient of the cross-linked aggregates versus the square of the scattering vector $q$ reveals a slight angular dependence (Figure IV-17B). The slight angular dependence suggests that the scattering comes from Brownian diffusion of particles with a heterodisperse distribution of sizes. Extrapolating to 0°, a $D_h$ value of 201 nm is
calculated using the Stokes-Einstein equation. Coincidentally, the $D_h$ was also measured at $90^\circ$ and found to be 177 nm, consistent with the Malvern instrument. Typically, the contributions of larger particles are suppressed at higher angles, and this is especially true for block copolymer vesicle formation. Static light scattering was also performed on the AuNP-“locked” solutions in order to determine the radius of gyration ($R_g$) from the angular dependence of the scattering intensity (Figure IV-17C). A plot of the inverse of the measured scattering intensity ($I_{ex}$) versus the square of the scattering vector $q$ provides a linear relationship leading to calculation of an $R_g$ value of 98 nm. This value along with the radius of hydration extrapolated to $0^\circ$ leads to an $R_g/R_h$ ratio of 0.98 which is indicative of a vesicular structure.\textsuperscript{94, 257, 258} TEM micrographs of the vesicles cross-linked by AuNP formation are shown in Figure IV-18A.

![TEM micrographs](image)

**Figure IV-18.** (a) AuNP cross-linked polymersomes formed from P(DMAEMA\textsubscript{165}-b-NIPAM\textsubscript{435}) and AuNP formed 48 h after addition of (b) cysteamine and (c) PEG-SH to the AuNP cross-linked polymersomes.

**Ligand Exchange to Reverse AuNP Cross-Linking of Vesicles**

Previous studies have shown that thiolated ligands are capable of displacing amine-containing, polymeric stabilizing agents on the surface of AuNPs.\textsuperscript{259, 260} Since thiolated ligands should have a stronger affinity for the gold surface than the amine functionalities along the DMAEMA block,\textsuperscript{261} the addition of either a small molecule
thiol, cysteamine, or a polymeric thiol, PEG-SH, should result in ligand exchange on the surface of the AuNPs leading to the disassembly of the cross-linked vesicles and subsequent binding of the AuNPs by the thiolated ligands. After allowing the ligand exchange reaction with cysteamine and PEG-SH to proceed for 48 h, the free polymer was removed by centrifugation and DLS and TEM measurements were conducted to determine the size and morphology of the thiolated AuNPs. The hydrodynamic diameters measured from DLS after the ligand exchange reaction with cysteamine and PEG-SH are shown in Figure IV-17A as curves c and d, respectively. For the reaction with cysteamine, the hydrodynamic diameter of the stabilized AuNPs is 16.0 nm, while the AuNPs stabilized by PEG-SH are slightly larger (20.4 nm), presumably due to the increased thickness of the PEG layer as compared to the bound cysteamine. Of note is the occurrence of a peak corresponding to residual AuNP cross-linked vesicles in both curves c and d, indicating that the ligand exchange was not quantitative within 48 h. Further experiments have shown that extending the reaction to longer times, however, does indeed lead to complete disappearance of the residual cross-linked peak. TEM micrographs of both systems (Figure IV-18 B and C) show near identical sizes of the resulting AuNPs of ~ 8 nm after the ligand exchange reactions. UV-vis spectroscopy was also used to follow the ligand exchange process by monitoring the absorbance before and after reaction. The AuNP-“locked” vesicles display the absorbance typically observed for AuNPs in aqueous solution with a $\lambda_{\text{max}}$ of 522 nm (Figure IV-19). Similarly, the UV-vis absorption spectra of the cysteamine and PEG-SH stabilized AuNPs show the characteristic absorbance attributed to the surface plasmon resonance of the AuNPs.
Reversible Shell Cross-Linking of AuNP-“locked” Micelles

P(DMAEMA<sub>165-b-NIPAM</sub><sub>102</sub>) (0.01wt%, pH 7.0) was utilized to form AuNP cross-linked micelles as discussed previously. Fixed angle DLS was utilized to follow the reversible crosslinking. At 50 °C, P(DMAEMA<sub>165-b-NIPAM</sub><sub>102</sub>) self assembled into micelles with a D<sub>h</sub> of 43 nm (Figure IV-20, curve a). After reduction of the solution temperature to 25 °C, the micelles remained intact and increased in size to 55 nm (Figure IV-20, curve b). A TEM micrograph (Figure IV-21A) of the AuNP cross-linked micelles shows spherical particles with diameters of approximately 5 nm. Treatment of the AuNP cross-linked micelles with the two thiols cysteamine and PEG-SH (10:1 DMAEMA:SH) resulted in the exchange of the DMAEMA units bound to the surface of the AuNPs and lead to the dissociation of the micellar structure. After removal of the liberated P(DMAEMA<sub>165-b-NIPAM</sub><sub>102</sub>), the AuNPs stabilized by cysteamine had an apparent D<sub>h</sub> of 6 nm (Figure IV-20, curve c) and Those stabilized by PEG-SH had a D<sub>h</sub> of 12 nm. (Figure IV-20, curve d). TEM micrographs of the cysteamine- and PEG-SH-stabilized AuNPs (Figures IV-21 B and C, respectively) show similar size particle of roughly 5 nm,
slightly smaller than the AuNPs formed in the shell of the vesicles discussed above. This revelation may open the door to templating AuNPs in nanostructures of varying sizes.

**Scheme IV-6.** Reversible AuNP-“locking” of P(DMAEMA$_{165}$-b-NIPAM$_{102}$) Micelles Accomplished by a Ligand Exchange of PDMAEMA for a Thiolated Stabilizing Agent.

**Figure IV-20.** DLS measurements showing the reversibility of the AuNP-“locking” of micelles formed from P(DMAEMA$_{165}$-b-NIPAM$_{102}$). (a) 0.01 wt% P(DMAEMA$_{165}$-b-NIPAM$_{102}$) (pH 7.0, T = 50 °C), (b) AuNP cross-linked micelles (T = 25 °C), AuNPs after ligand exchange with (c) cysteamine and (d) PEG-SH.
Figure IV-21. (a) AuNP cross-linked micelles formed from \( \text{P(DMAEMA}_{165}\text{-}b\text{-NIPAM}_{102}) \) and AuNP formed 48 h after addition of (b) cysteamine and (c) PEG-SH to the AuNP cross-linked micelles.
Section IV. “Schizophrenic” Self-Assembly of Block Copolymers Synthesized via Aqueous RAFT Polymerization: From Micelles to Vesicles

Overview

In a previous section, the ability to tune the solution morphology of a series of block copolymers of DMAEMA (M17) and NIPAM (M1) was discussed. The complex stimuli-responsive behavior of DMAEMA lead to the manipulation of copolymer composition, solution pH, temperature, and ionic strength to control the hydrophilic mass fraction and hence dictate self-assembled morphology. Incorporation of a block which undergoes a pH-responsive hydrophilicity change into a diblock copolymer with NIPAM allows for not only the ability to tune the hydrophobic content of the block copolymer but also allows for the investigation of “schizophrenic” aggregation behavior under the influence of two disparate stimuli. Herein, we describe the RAFT synthesis and solution behavior of two block copolymers of N,N-diethylaminoethyl methacrylate (DEAEMA) (M19) and NIPAM specifically designed to elucidate the relationship between hydrophilic mass fraction and the resulting solution morphology. By adjusting the pH and temperature, PDEAEMA and PNIPAM blocks were respectively rendered hydrophobic. Aqueous RAFT polymerization was utilized to ensure the synthesis of well-defined block copolymers with narrow PDIs. This work represents the first example, to our knowledge, of double hydrophilic block copolymers exhibiting a morphological transition from micelles to vesicles based on stimuli-responsive behavior.

Synthesis of Block Copolymers of DEAEMA and NIPAM

RAFT provides a facile technique for preparing well-defined amphiphilic diblock copolymers of preselected compositions to test the effect of block lengths (i.e.
hydrophilic weight fraction) on the self-assembled morphology in aqueous solution. The present diblock copolymer system was chosen due to the pH-response of PDEAEMA (pKa ~ 7.3)\textsuperscript{262} and the thermoresponse of PNIPAM (LCST ~ 32 °C). Specific copolymer compositions were targeted to produce hydrophilic mass fractions for “schizophrenic” micelle-to-unimer-to-micelle (Scheme IV-6A) and micelle-to-unimer-to-vesicle (Scheme IV-6B) transitions according to Discher’s and Eisenberg’s empirical relationship.\textsuperscript{163} The diblock copolymers of DEAEMA and NIPAM were synthesized according to Scheme III-2. The trithiocarbonate, CEP (CTA\textsubscript{8}), was used to mediate the aqueous RAFT polymerization of DEAEMA (M\textsubscript{19}) in the presence of the free radical initiator V-501 (I\textsubscript{3}) to yield PDEAEMA\textsubscript{98} (P\textsubscript{7}) (M\textsubscript{n} = 18.4 kDa, PDI = 1.07). The PDEAEMA\textsubscript{98} macroCTA was subsequently chain extended with NIPAM (M\textsubscript{1}) to produce two diblock copolymers. The diblock copolymers were targeted to have 50 and 70 wt% NIPAM. The two diblock copolymers, P(DEAEMA\textsubscript{98}-b-NIPAM\textsubscript{209}) (P\textsubscript{8}) (M\textsubscript{n} = 39.3 kDa, PDI = 1.08) and P(DEAEMA\textsubscript{98}-b-NIPAM\textsubscript{392}) (P\textsubscript{9}) (M\textsubscript{n} = 63.9 kDa, PDI = 1.10), were determined to have 53.4 and 71.4 wt% NIPAM, respectively, using SEC (Figure IV-22). \textsuperscript{1}H NMR studies of the two diblock copolymers revealed weight fractions (52.5 and 70.8 wt%) in agreement with those determined by SEC. SEC chromatograms of PDEAEMA\textsubscript{98}, P(DEAEMA\textsubscript{98}-b-NIPAM\textsubscript{209}), and P(DEAEMA\textsubscript{98}-b-NIPAM\textsubscript{392}) were unimodal and the PDIs were low (< 1.2) indicating near-quantitative blocking efficiency and controlled polymerization. The molecular weight and composition data of the diblock copolymer series are summarized in Table IV-3.
Scheme IV-7. Representation of Proposed “Schizophrenic” Aggregation Behavior for (a) P(DEAEMA\textsubscript{98}−b-NIPAM\textsubscript{209}) and (b) P(DEAEMA\textsubscript{98}−b-NIPAM\textsubscript{392}).

Figure IV-22. SEC chromatograms for (a) PDEAEMA\textsubscript{98}, (b) P(DEAEMA\textsubscript{98}−b-NIPAM\textsubscript{209}), and (c) P(DEAEMA\textsubscript{98}−b-NIPAM\textsubscript{392}).
**Scheme IV-8.** Synthesis of Dually-Responsive Block Copolymers of DEAEMA and NIPAM via Aqueous RAFT Polymerization.

**Table IV-3.** Summary of P(DEAEMA$_{98}$-$b$-NIPAM$_x$) molecular weights and compositions.

<table>
<thead>
<tr>
<th></th>
<th>$M_n$ (kDa)$^a$</th>
<th>PDI$^a$</th>
<th>wt% (mol%) NIPAM$^b$</th>
<th>wt% (mol%) NIPAM$^b$</th>
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</thead>
<tbody>
<tr>
<td>PDEAEMA$_{98}$ (P7)</td>
<td>18.4</td>
<td>1.07</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P(DEAEMA$<em>{98}$-$b$-NIPAM$</em>{209}$) (P8)</td>
<td>39.3</td>
<td>1.08</td>
<td>53.4 (65.4)</td>
<td>52.5 (64.4)</td>
</tr>
<tr>
<td>P(DEAEMA$<em>{98}$-$b$-NIPAM$</em>{392}$) (P9)</td>
<td>63.9</td>
<td>1.10</td>
<td>71.4 (80.4)</td>
<td>72.4 (81.1)</td>
</tr>
</tbody>
</table>

$^a$ As determined by SEC. $^b$ As determined by $^1$H NMR.

“Schizophrenic” Self-Assembly of P(DEAEMA$_{98}$-$b$-NIPAM$_{209}$)

Block copolymers of DEAEMA and NIPAM are expected to undergo “schizophrenic” aggregation behavior due to the separate responsive behaviors exhibited by the two blocks. $^1$H NMR was utilized to investigate the dual responsiveness of the two DEAEMA and NIPAM block copolymers in aqueous solution. Figure IV-23 shows the temperature- and pD-dependent $^1$H NMR spectra for P(DEAEMA$_{98}$-$b$-NIPAM$_{209}$) (P8) (0.01 wt%) dissolved in D$_2$O. At 25 °C and pD 5.0, the diblock copolymers are expected to exist as unimers, since the conditions are below the pK$_a$ of the PDEAEMA
block and below the LSCT of the PNIPAM block. In the $^1$H NMR spectrum of Figure IV-23A, the characteristic resonances of PDEAEMA (a, b, and c) and the characteristic resonance of PNIPAM (d) are readily visible. Increasing the pD to a value of 9.0 leads to deprotonation and hydrophobic collapse of the PDEAEMA block, as evidenced by the attenuation of peaks a, b, and c associated with PDEAEMA while the PNIPAM peak d is still present. Conversely, at 50 °C and pD 5.0, the peak for the PNIPAM is attenuated and the PDEAEMA peaks are seen. While the $^1$H NMR experiments provide evidence for the “schizophrenic” self-assembly behavior, conclusions as to the aggregate morphology cannot be made from these data. In order to investigate the effect of solution pH and temperature on morphological transitions, a combination of DLS and SLS as well as electron microscopy was utilized.

![Diagram of molecular structure]

**Figure IV-23.** $^1$H NMR spectra of P(DEAEMA$_{98}$-b-NIPAM$_{209}$) (0.1 wt%) at (A) 25 °C and pD 5.0, (B) 25 °C and pD 9.0, and (C) 50 °C and pD 5.0.

The stimuli-responsive behavior of P(DEAEMA$_{98}$-b-NIPAM$_{209}$) was additionally investigated using DLS. Figure IV-24A shows the temperature- and pH-responsiveness
of P(DEAEMA$_{98}$-b-NIPAM$_{209}$) (0.01 wt%) in aqueous solution. Under these conditions, P(DEAEMA$_{98}$-b-NIPAM$_{209}$) exists as unimers of ~ 10 nm at pH 5.0 and 25 °C (Figure IV-24B, curve a). At pH values above the pK$_a$ of PDEAEMA, P(DEAEMA$_{98}$-b-NIPAM$_{209}$) self-assembles into aggregates with hydrodynamic diameters of ~ 40 nm (Figure IV-24B, curve b). In order to ensure the PDEAEMA block remains protonated, and therefore hydrophilic, the thermoresponsive self-assembly of P(DEAEMA$_{98}$-b-NIPAM$_{209}$) was studied at a solution pH of 5.0. At temperatures above 42 °C, this diblock copolymer formed aggregates of sizes between 50 and 65 nm. The size of these aggregates decreased with increasing temperature above the CAT which can be attributed to increasing dehydration of the PNIPAM block. At 50 °C, P(DEAEMA$_{98}$-b-NIPAM$_{209}$) formed aggregates of 52.2 nm (Figure IV-24B, curve c). While DLS at a single angle allows for determination of hydrodynamic size of the nanoassemblies, it does not provide information on aggregate morphology.

Figure IV-24. (A) Responsive aggregation behavior of P(DEAEMA$_{98}$-b-NIPAM$_{209}$) (0.01 wt%) at (●) 25 °C and variable pH and (■) pH 5.0 and variable temperature. (B) Hydrodynamic diameter of P(DEAEMA$_{98}$-b-NIPAM$_{209}$) (0.01 wt%) at (a) 25 °C and pH 5.0, (b) 25 °C and pH 9.0, and (c) 50 °C and pH 5.0.
In order to study the nature of the aggregate structure, variable angle DLS and SLS were used in combination with electron microscopy. The angular dependent DLS and SLS results for aggregates formed from P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) at 25 °C and pH 9.0 are shown in Figure IV-25A. A plot of the relaxation frequency (Γ) versus the square of the scattering vector (q<sup>2</sup>) gives a linear relationship, indicative of Brownian diffusion of spherical particles. The slope through the origin yields a diffusion coefficient of 1.153 x 10<sup>-11</sup> m<sup>2</sup>/s. Using the Stokes-Einstein equation, an apparent hydrodynamic radius (R<sub>h</sub>) of 21.2 nm was determined, which is in good agreement with measurements taken at a fixed angle. A radius of gyration (R<sub>g</sub>) of 16.4 nm was calculated using a Zimm treatment of the SLS data. The ratio of R<sub>g</sub>/R<sub>h</sub> determined from angular dependent DLS and from SLS for the self-assembled aggregates of P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) at 25 °C and pH 9.0 is 0.774, which is indicative of hard-sphere particles. The formation of spherical particles under identical conditions was also confirmed by TEM (Figure IV-26A). By utilizing a combination of techniques (¹H NMR, light scattering, and TEM), the aggregate morphology of each system has been elucidated. P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) self-assembles into PDEAEMA-core, PNIPAM-shell spherical micelles at 25 °C and pH 9.0. Furthermore, LS experiments of P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) at 50 °C and pH 5.0 (Figure IV-25B) indicated R<sub>h</sub>, R<sub>g</sub>, and R<sub>g</sub>/R<sub>h</sub> values of 28.3 nm, 21.6 nm, and 0.763, respectively. These values along with ¹H NMR (Figure IV-23C) and TEM (Figure IV-26B) measurements support the formation of PNIPAM-core, PDEAEMA-shell spherical micelles.
Figure IV-25. Angular dependent DLS (■) and SLS (●) measurements performed on P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) (0.01 wt%) at (A) 25 °C and pH 9.0 and (B) 50 °C and pH 5.0.

Figure IV-26. TEM micrographs of P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) (0.01 wt%) at (a) 25 °C and pH 9.0 and (b) 50 °C and pH 5.0.

Table IV-4. Summary of Light Scattering data for P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>).

<table>
<thead>
<tr>
<th>pH</th>
<th>T (°C)</th>
<th>R&lt;sub&gt;g&lt;/sub&gt; (nm)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R&lt;sub&gt;h&lt;/sub&gt; (nm)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R&lt;sub&gt;g&lt;/sub&gt;/R&lt;sub&gt;h&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R&lt;sub&gt;g&lt;/sub&gt;/R&lt;sub&gt;h&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>5.0</td>
<td>25</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.0</td>
<td>25</td>
<td>16.4</td>
<td>20.6</td>
<td>21.2</td>
<td>0.796</td>
</tr>
<tr>
<td>5.0</td>
<td>50</td>
<td>21.6</td>
<td>26.1</td>
<td>28.3</td>
<td>0.828</td>
</tr>
</tbody>
</table>

<sup>a</sup> Measured using Malvern Instruments Zetasizer Nano. <sup>b</sup> Measured using a Spectra Physics Millenia laser in conjunction with a Brookhaven BI-200SM goniometer with a BI-9000 correlator.
"Schizophrenic" Self-Assembly of P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>)

The second diblock copolymer, P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>) (P9), was designed such that the self-assembly into micelles would occur under conditions rendering the PDEAEMA block hydrophobic and vesicles when the PNIPAM block was hydrophobic. Fixed angle DLS was used to study the effects of solution pH and temperature on the size of the self-assembled aggregates. As observed for P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>), a plot of hydrodynamic size versus solution pH (Figure IV-27A) revealed a transition from unimers of ~ 14 nm (Figure IV-27B, curve a) to aggregates of ~ 53 nm above the pK<sub>a</sub> of PDEAEMA at 25 °C (Figure IV-27B, curve b). The temperature-responsive self assembly of P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>) was analyzed at pH 5.0 to ensure that the PDEAEMA segments remained hydrophilic. The CAT of P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>) is 38 °C, which is lower than that observed for P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>). This has been attributed to the increased hydrophobic content in the diblock copolymer. At 38 °C, P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>) self-assembled into aggregates of 215 nm. The size of the aggregates decreased with increasing temperature as observed for the micelles formed from P(DEAEMA<sub>98</sub>-b-NIPAM<sub>209</sub>) above the CAT. At 50 °C, P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>) self-assembles into aggregates of 199 nm (Figure IV-27B, curve c).
Angular dependent DLS and SLS were also utilized to investigate the observed morphology of P(DEAEMA$_{98}$-b-NIPAM$_{392}$) under various solution conditions. At temperatures below the LCST of PNIPAM, when the pH is increased above the pK$_a$ of PDEAEMA, the diblock copolymer is 70.8 wt% hydrophilic, and should aggregate to form spherical micelles according to the empirical relationship proposed by Discher and Eisenberg.$^{163}$ Figure IV-28A shows the LS analysis of P(DEAEMA$_{98}$-b-NIPAM$_{392}$) at 25 °C and a solution pH of 9.0. Multi-angle DLS measurements yield an apparent diffusion coefficient and an R$_h$ value of $9.625 \times 10^{-12}$ m$^2$/s and 25.4 nm, respectively. An R$_g$ of 21.1 nm is measured using SLS yielding an R$_g$/R$_h$ value of 0.793, indicative of spherical micelles.$^{257, 258, 264}$ TEM also confirms the formation of spherical micelles (Figure IV-29A) from P(DEAEMA$_{98}$-b-NIPAM$_{392}$) at 25 °C and a solution pH value of 9.0. When the solution pH is maintained at 5.0 to ensure that PDEAEMA is hydrophilic, the solution temperature can be raised above the LCST of PNIPAM to induce self-assembly. Under these conditions, P(DEAEMA$_{98}$-b-NIPAM$_{392}$) has a hydrophilic mass fraction of 29.2
wt% and should self-assemble into vesicles. Angular-dependent DLS and SLS (Figure IV-28B) reveal apparent $R_h$ and $R_g$ values of 91.5 and 99.2 nm, respectively. The ratio of $R_g/R_h$ (1.08) corresponds well to the theoretical value for vesicles (1.0).\textsuperscript{257, 258, 264} TEM micrographs of samples stained with phosphotungstic acid confirm structures with the characteristic vesicular structure (Figure IV-29B). Note that the $\Gamma$ vs. $q^2$ plots remain linear, indicating that the spherical morphology is retained over the pH and temperature range.

**Figure IV-28.** Angular dependent DLS (■) and SLS (●) measurements performed on P(DEAEMA\textsubscript{98}-b-NIPAM\textsubscript{392}) (0.01 wt%) at (A) 25 °C and pH 9.0 and (B) 50 °C and pH 5.0.

**Figure IV-29.** TEM micrographs of P(DEAEMA\textsubscript{98}-b-NIPAM\textsubscript{392}) (0.01 wt%) at (a) 25 °C and pH 9.0 and (b) 50 °C and pH 5.0.
Table IV-5. Summary of Light Scattering Data for P(DEAEMA<sub>98</sub>-b-NIPAM<sub>392</sub>).

<table>
<thead>
<tr>
<th>pH</th>
<th>T (°C)</th>
<th>$R_g$ (nm)</th>
<th>$R_h$ (nm) Malvern</th>
<th>$R_h$ (nm) Brookhaven</th>
<th>$R_g/R_h$ Malvern</th>
<th>$R_g/R_h$ Brookhaven</th>
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</thead>
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<tr>
<td>9.0</td>
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<td>26.6</td>
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<td>98.8</td>
<td>91.4</td>
<td>1.00</td>
<td>1.08</td>
</tr>
</tbody>
</table>

CHAPTER V

CONCLUSIONS

Section I. *In Situ* Formation of Gold-“Decorated” Vesicles from a RAFT-synthesized, Thermally Responsive Block Copolymer

In summary, thermally responsive vesicles have been prepared from the self-assembly of $\text{P(DMAEMA}_{73-b\text{-PNIPAM}}_{99})$ (P2). Simply mixing the vesicle solution with a solution of NaAuCl$_4$, without the necessity of an external reducing agent, leads to the formation of gold nanoparticle decorated vesicles. Based on our studies thus far, we postulate a sequence of events which may account for formation and “locking” of gold nanoparticle-decorated vesicles reported in this manuscript. Thermally driven vesicle formation from unimers occurs above the LCST of responsive NIPAM (M1) block. Mixing the polymer solution with NaAuCl$_4$ allows counterion exchange with the protonated DMAEMA (M17) polyelectrolyte segments. Subsequent *in situ* reduction to zero-valent gold occurs, perhaps induced by the small number of unprotonated amines present at the reaction pH. The conversion of complexed AuCl$_4^-$ to zero-valent gold nanoparticles is confirmed by the observed surface plasmon resonance.
Section II. Tuning Nanostructure Morphology and Gold Nanoparticle “Locking” of Multi-Responsive Amphiphilic Diblock Copolymers

In this work, we have described the facility by which hydrophilic-hydrophilic diblock copolymers can be synthesized and induced to undergo stimuli-responsive reorganization into nano-aggregates with specific morphology. Three block copolymers of DMAEMA (M17) and NIPAM (M1) with a fixed PDMAEMA length of DP=165 and PNIPAM blocks of 102 (P4), 202 (P5), and 435 (P6) have been successfully synthesized via RAFT polymerization. It was shown that decreasing the hydrophilic mass fraction of the block copolymers through changes in composition, pH, or ionic strength drastically affects the resulting assembly behavior and morphology. By carefully controlling these parameters, spherical micelles, worm-like micelles, and vesicles were prepared from the stimuli-responsive, hydrophilic-hydrophilic block copolymers directly in water. Significantly, these amphiphilic diblock copolymers subjected to external stimuli behave as predicted from theory developed by Discher, Eisenberg, and others for amphiphilic diblocks with a permanently hydrophobic block. The nanostructures were subsequently cross-linked to yield AuNPs by the in situ reduction of NaAuCl₄ by the amine moieties in the PDMAEMA shells and observed by TEM. Importantly, the ability of stimuli-responsive hydrophilic-hydrophilic block copolymers to assemble directly in aqueous media provides important pathways for biologically relevant applications.
Section III. Reversible AuNP Shell Cross-linking of Nanostructures Derived from Stimuli-Responsive Diblock Copolymers

In summary, we have demonstrated a facile method for reversing the AuNP cross-linking of aggregates self-assembled from RAFT-generated polymers. Polymersomes self-assembled from thermally-responsive P(DMAEMA$_{165}$-b-NIPAM$_{435}$) (P$_6$) block copolymers were prepared and cross-linked with AuNPs utilizing our previously reported procedure.\textsuperscript{265} Employing ligand exchange reactions, the DMAEMA units bound to the surface of the in situ formed AuNPs were displaced by the smaller, stronger binding thiols, reversing the cross-links formed in the shell of the micelles and vesicles. This reversible cross-linking method may prove useful for the preparation and eventual degradation of AuNP-“locked” theranostic vehicles targeting cancerous tissue where thiol concentrations can be as high as 7 times those in surrounding tissue.\textsuperscript{266, 267}
Section IV. “Schizophrenic” Self-Assembly of Block Copolymers Synthesized via Aqueous RAFT Polymerization: From Micelles to Vesicles

The aqueous RAFT synthesis and characterization of dually-responsive diblock copolymers of DEAEMA (M19) and NIPAM (M1) capable of “schizophrenic” aggregation into multiple morphologies are described. The two diblock copolymers were specifically designed to test the empirical relationship proposed by Discher and Eisenberg\(^{163}\) correlating the hydrophilic mass fraction to the resultant self-assembled solution morphology. The smaller block copolymer, P(DEAEMA\(_{98-b}\)-NIPAM\(_{209}\)) (P8) (52.5 wt% NIPAM), assembled into a) spherical PDEAEMA-core micelles below the LCST of PNIPAM and above the pK\(_a\) of PDEAEMA and b) spherical PNIPAM-core micelles above the LCST of PNIPAM and below the pK\(_a\) of PDEAEMA. The larger block copolymer, P(DEAEMA\(_{98-b}\)-NIPAM\(_{392}\)) (P9) (70.8 wt% PNIPAM), was designed to be asymmetric and capable of assembly into micelles at high pH and vesicles at high temperature. At 25 °C and pH > 7.5, P(DEAEMA\(_{98-b}\)-NIPAM\(_{392}\)) was shown to assemble into PDEAEMA-core micelles, whereas at pH 5.0 and temperatures above the CAT, vesicles were formed. To our knowledge, this represents the first report of a block copolymer system capable of a “schizophrenic” micelle-to-unimer-to-vesicle morphological transition in aqueous solution in response to multiple stimuli.
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