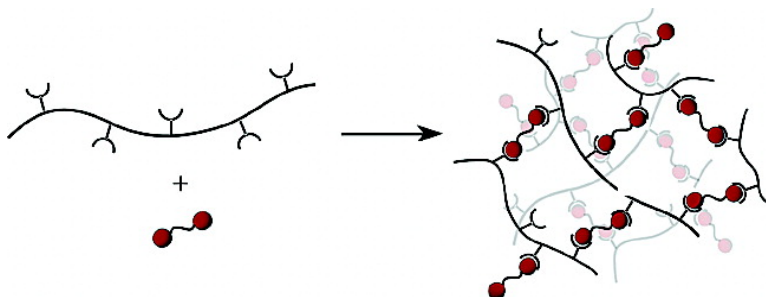**Article**

Thermally Reversible Formation of Microspheres through Non-Covalent Polymer Cross-Linking

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Thermally Reversible Formation of Microspheres through Non-Covalent Polymer Cross-Linking

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Abstract: Bis-thymine units were used to noncovalently cross-link a complementary diamidopyridine-functionalized copolymer. Upon combination in noncompetitive solvents, discrete micron-scale spherical aggregates were formed arising from specific three-point polymer-cross-linker hydrogen bonding interactions. The diameter of these microspheres could be controlled through spacer structure. The cross-linking process was fully thermally reversible, with complete dissolution observed at 50 °C and reformation of the aggregates upon return to ambient temperature. This process could be repeated multiply, with lower particle dispersity observed arising from the annealing process.

Introduction

Molecular self-assembly through specific interactions is a versatile tool for the thermodynamically controlled generation of higher-order structures.[1] Application of this strategy to macromolecular systems including dendrimers[2] and polymers[3] extends the utility of this methodology for the creation of materials. Controlled polymer aggregation through phase separation has been used to create a diverse array of structures, including gels,[4] micelles,[5] vesicles,[6] and other spherical and nonspherical aggregates.[7] These structures have numerous potential applications ranging from encapsulation and drug delivery[8] to nano- and micron-sized reaction vessels.[9] Combination of polymer aggregation with the reversible nature of molecular recognition processes furnishes an attractive means of constructing recyclable materials responsive to thermal,[10] electrochemical,[11] or photochemical stimulus.[12]

We recently reported the post-polymerization modification of *poly*-styrene-*co*-4-chloromethylstyrene with randomly dispersed thymine (THY) and diacyldiamidopyridine (DAP) functionalities.[13] Upon combination in noncompetitive solvents, giant

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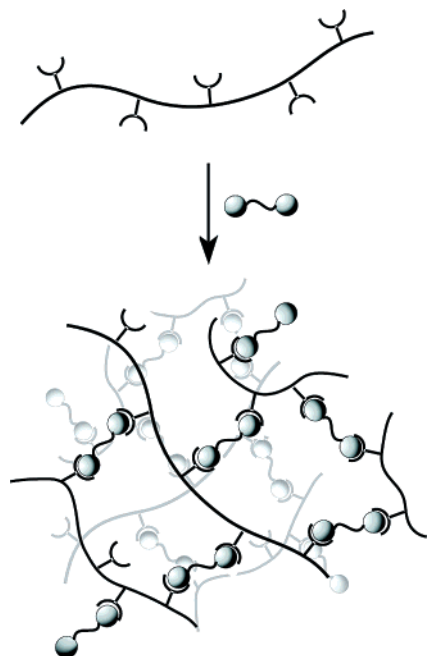
Figure 1. Schematic illustration of noncovalent polymer cross-linking of copolymers using complementary bifunctional cross-linkers.

vesicles (recognition-induced polymersomes (RIPs)) were formed due to the highly specific interactions between complementary side chains on polymer backbones.[14] To provide an alternate motif for noncovalent cross-linking (Figure 1), we have synthesized a series of bis-thymines[15] that are complementary to the diamidopyridine side chains of polymer **1** (Figure 2). We report here the thermoreversible formation of micron-scale spherical aggregates using these systems.

Results and Discussion

Mixing of polymer **1** in CHCl₃ with one equivalent (based on recognition element) of each of the cross-linkers resulted in a turbid solution arising from suspended aggregates. This aggregation process arose from specific intermolecular interactions: no turbidity was observed with **NMe 8c**, a highly analogous system that cannot participate in three-point hydrogen bonding. The structure of the aggregates was established by Laser Confocal Scanning Microscopy (LCSM) using flavin-tagged polymer **1a**.[16] CLSM images showed that well-defined, micron-sized spherical aggregates (Figure 3) were formed from combination of polymer **1a** with each of the cross-linkers. The solid fluorescence profile of these spherical assemblies is indicative of a filled sphere rather than the vesicular morphology observed in our previous studies.[14] In all micrographs, fluorescence is more intense in the aggregate center suggesting a microgel morphology. The highly localized fluorescence observed in the micrographs indicates that very little flavin-tagged polymer **1a** exists free in solution, demonstrating the efficiency of the cross-linking process.
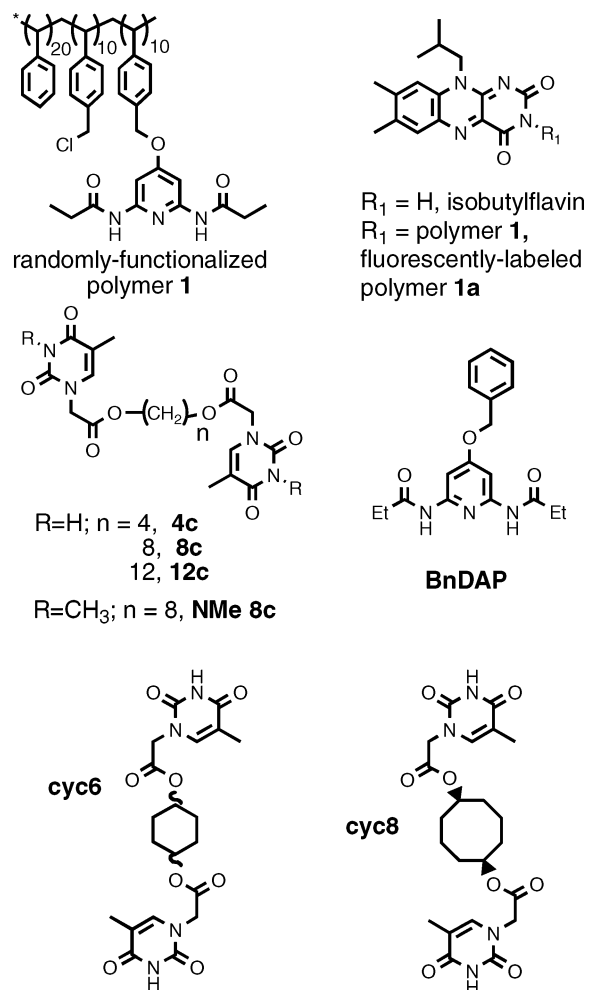
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(16) LCSM allows for the visualization of thin aggregate cross-sections (∼100 nm) that provide compelling evidence of internal aggregate structure.

Figure 2. Diamidopyridine-based (DAP) polymer **1**, bis-thymine cross-linkers, and non-hydrogen bonding control **NMe8c**.
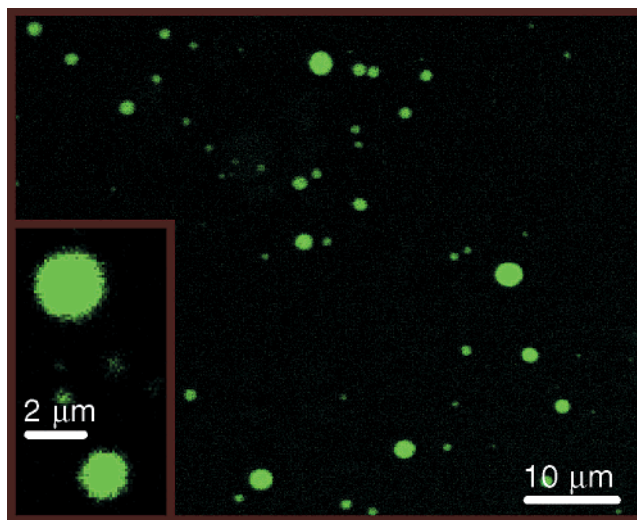

Figure 3. Representative LCSM images of **8c** combination with polymer **1**. Inset shows cross sections of microspheres indicating filled structures. Other cross-linker-polymer **1** combinations generated similar micrographs.

The as-formed microspheres are quite stable, existing in solution for weeks with no precipitation. For this reason, it is believed that these aggregates are discrete microgels, or solvent-swollen cross-linked networks. Gels forms at the air−liquid interface when the system is allowed to sit undisturbed (>6 h),
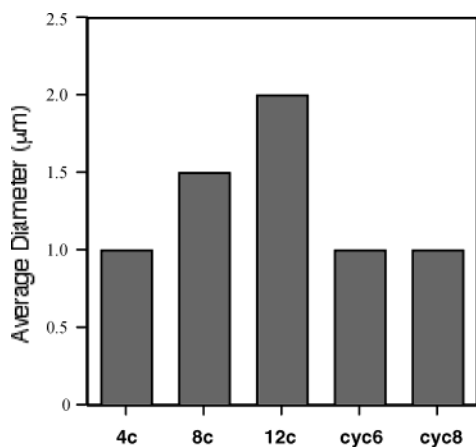
Figure 4. Median diameters of spherical aggregates obtained from complexation of polymer **1** with each bisfunctional thymine cross-linker.

presumably due to interdigitation of the polymers comprising the aggregates. This macrogel, however, can be easily redispersed by short periods (5–10 s) of agitation. At higher component concentrations, it is believed that this interdigitation would be observed throughout the system leading to formation of a gel, but poor cross-linker solubility in noncompetitive solvents limits study in this direction. As expected, reducing the concentration of each component, while maintaining the optimum 1:1 DAP:THY ratio drastically affects the assembly process. Cross-linker concentrations at and below 750 mg/mL (only 75% of concentrations employed throughout the following study) produce no observable aggregates on the micron scale. For this reason, more concentrated solutions of cross-linkers were utilized to provide optimal size and distribution of microspheres.

Differential Interference Microscopy (DIC) was used to obtain size distributions for the aggregates formed using each cross-linker.[17] With cross-linkers **4c**, **8c**, and **12c** featuring flexible spacers, a monotonic increase in median size of the resulting microspheres was observed with increasing chain length (Figure 4).[18] The more rigid cycloaliphatic cross-linkers **cyc6** and **cyc8** provide aggregate size distributions similar to that of microspheres comprised of the shortest cross-linker **4c** and polymer **1**, suggesting this size dependence may derive from the degree of linker preorganization.

Further insight into the assembly process was obtained through ^{1}H NMR titration (Figure 5). These data show the chemical shift of the imide proton of the thymine cross-linker move downfield between 3.0 and 4.8 ppm due to specific three-point hydrogen bonding. Consistent with a specific recognition process, saturation is observed in each case. Also, each cross-linker displays similar binding behavior, differing in the final chemical shift of the imide functionality. The data, however, cannot be accurately fit to a discrete 1:1 binding isotherm due
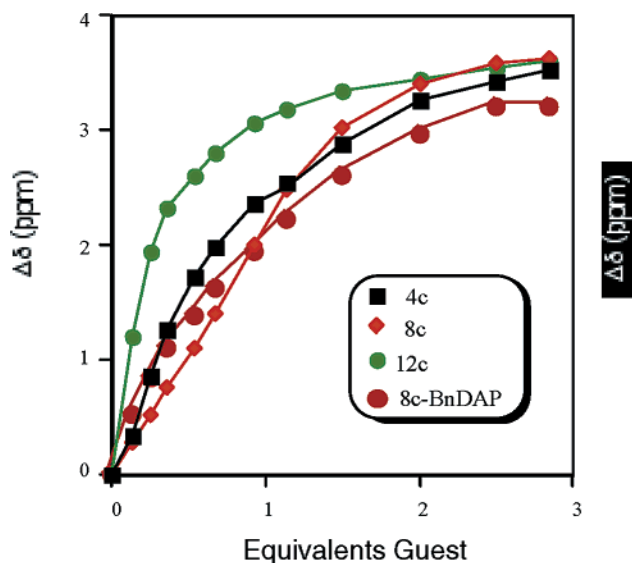
(17) DIC is a phase-contrast microscopy that allows the user to observe translucent structures. DIC has the additional benefit of facile accumulation of large numbers of micrographs. This allows for acquisition of an adequate sampling population that can be converted to accurate size distributions. Kachar, B.; Evans, D. F.; Ninham, B. W. *J. Colloid Interface Sci.* **1984**, *100*, 287–301. Prentice, P.; Hashemi, S. *J. Mater. Sci.* **1984**, *19*, 518–526.

(18) From the size distributions depicted in Figure 4, it can be inferred that submicron assemblies exist within this system that are below the resolution of optical microscopy. Distortion that occurs with higher resolution techniques such as Transmission and Scanning Electron Microscopies limit the utility of these methods for assessing solution state properties for these systems.

Figure 5. Curves generated from ^{1}H NMR titrations of various cross-linkers with complementary polymer. For each aggregate formed, the bisthymine cross-linker was employed as the host molecule (5.0×10^{-3} M recognition element) with the imide proton of the thymine moiety monitored; polymer was added as a solution in CDCl$_3$ (2.0×10^{-2} M recognition element). Curve fit is to lead the eye.
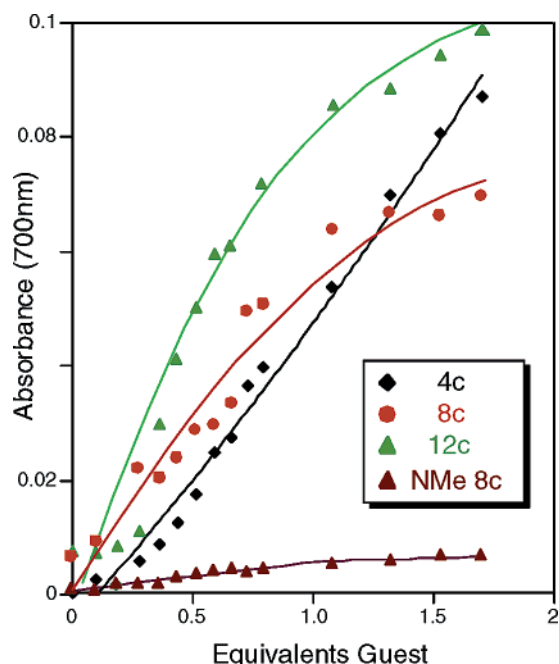

Figure 6. Noncovalent polymer association as monitored by solution turbidity at 700 nm in CHCl$_3$. [Cross-linker] = 5.00×10^{-4} M (based on recognition elements). Curve fit is to lead the eye.

to the complexity of the assembly process. Interestingly, titration of **8c** with benzylated DAP monomer, **BnDAP**, results in a slightly lower overall chemical shift than that of polymer **1**. This is in contrast to previous results where it was determined that covalent attachment of a recognition element to a polymeric backbone results in a drop in binding efficiency.[13] The larger change chemical shifts observed with each of the cross-linkers relative to **BnDAP** provides evidence for a distinctly different environment within the microsphere.

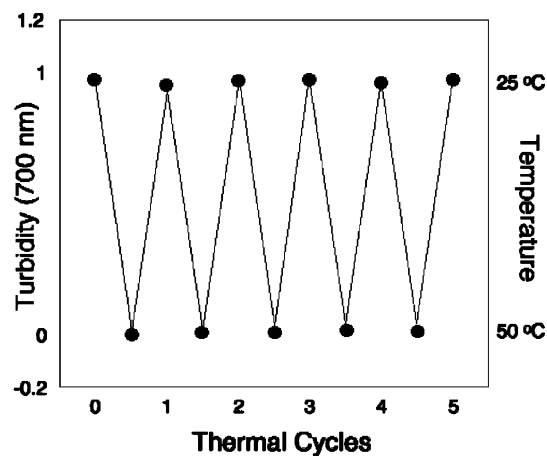Titrations designed to monitor solution turbidity were employed to correlate ^{1}H NMR experiments with the self-assembly

Figure 7. Solution turbidity (700 nm) of microspheres generated from **8c**-polymer **1** combination during heat-cool cycles.

Table 1. Average Diameters and Standard Deviations of Microspheres Before and After Thermal Cycles

	before heat cycles	after heat cycles
4c	2.1 ± 1.1	1.5 ± 0.7
8c	2.1 ± 1.5	1.7 ± 0.7
12c	2.6 ± 2.0	1.7 ± 0.6
Cyc6	2.0 ± 1.1	1.7 ± 0.9
Cyc8	2.1 ± 1.1	1.6 ± 0.6

process. Using the same polymer and cross-linker concentration, polymer association was followed by monitoring solution absorbance at 700 nm, a wavelength at which neither cross-linkers nor polymer **1** absorb. These spectra clearly show increasing solution turbidity as a function of added guest, indicating increasing aggregation of solution components. The rapid increase in solution turbidity also serves to demonstrate the high efficiency and cooperativity of this system. As expected, no turbidity was observed when **NMe 8c** was titrated with polymer **1**.

Thermal reversibility is one of the key attributes of self-assembled materials, making the resultant architectures both processable and recyclable, and providing potential leads for the development of delivery and transport systems.[19] The thermoreversibility of microsphere formation was probed using turbidometry: heating of the microspheres in a sealed vessel to 50 °C resulted in a total loss of turbidity, indicating complete dissolution of the aggregates (Figure 7). Upon cooling to room temperature (23 °C), microspheres spontaneously reformed, fully restoring the turbidity. This process could be repeated multiple times with little or no change of turbidity at either temperature.

Although little change was observed in turbidity upon thermal cycling, DIC microscopy revealed that the size distribution of the aggregates differed after the heating–cooling cycles. After cycling, the aggregates were slightly smaller on average, and much less polydisperse (Table 1). The pattern was observed

with every cross-linker-polymer combination. This change in distribution presumably arises from the annealing of the initial kinetically formed structures to thermodynamically more stable aggregates upon thermal cycling.

Conclusions

In summary, we have demonstrated the thermally reversible formation of micron-size gel-like spherical aggregates through noncovalent polymer cross-linking. This cross-linking occurred as a direct result of specific three-point hydrogen bonding between polymer side chains and bifunctional molecules featuring complementary functionality, as determined by parallel ^{1}H NMR and solution turbidity titrations. The spherical aggregates are stable indefinitely at ambient temperature, dissociate at 50 °C, and reform upon cooling; this heating–cooling cycle can be repeated multiply with no decomposition. Interestingly, thermal cycling results in the formation of more monodisperse particles after heat–cool cycles.

The thermal response coupled with the discrete aggregates formed using noncovalent cross-linking makes these systems applicable to many potential applications, including chemical storage, encapsulation, and transport systems. Investigations into the origin of phase segregation along with development of practical applications for these materials are currently underway and will be presented in due course.

Supporting Information Available: Complete experimental details, ^{1}H NMR and UV–vis spectra for polymer:cross-linker titrations and thermal reversibility experiments as well as size distributions for each cross-linker–polymer combination. This material is available free of charge via the Internet at http://pubs.acs.org.

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