

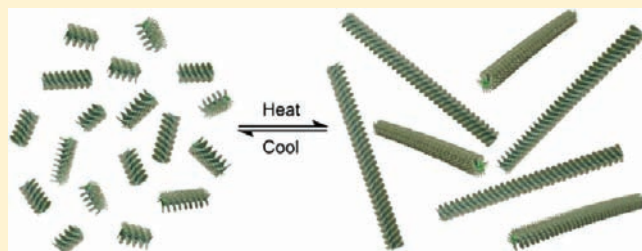
Thermoresponsive Dynamers: Thermally Induced, Reversible Chain Elongation of Amphiphilic Poly(acylhydrazones)

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S Supporting Information

ABSTRACT: A nanostructured poly(acylhydrazone), which is reversibly formed in acidic aqueous solution from di(aldehyde) and di(acylhydrazine) monomers with appended hexaglyme groups, was found to display lower critical solution (LCS) behavior. Remarkably, under acidic conditions in which polymerization is reversible, large and reversible molecular weight (M_w) increases were observed in response to elevated temperatures, both below and above the LCS temperature. No variation in M_w was evident under neutral and alkaline conditions, in which the acylhydrazone condensation is essentially irreversible. Results of turbidometry studies, size-exclusion chromatography–multiangle laser light scattering (SEC–MALLS), and transmission electron microscopy (TEM) suggest that heating the polymer below the LCS temperature leads to polymer growth with preservation of the characteristic nanostructured morphology, whereas the onset of the microphase separated state causes a fundamental change in morphology, in which the polymer chains aggregate into larger bundles and fibers. van't Hoff analysis of a small molecule model system indicates that the acylhydrazone condensation is enthalpy driven ($\Delta H_{eq} = -8.2 \pm 0.2 \text{ kcal} \cdot \text{mol}^{-1}$ and $\Delta S_{eq} = -11.1 \pm 0.4 \text{ cal} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$), which suggests that the observed polymer growth with temperature is not a consequence of the intrinsic thermodynamics of the intermonomer linkage but is likely the result of the thermoresponsive characteristics conferred by the multiple hexaglyme groups. The system described displays double control of the dyanmer state by two orthogonal agents, heat and protons (pH). It also represents a prototype for dynamic materials displaying multiple control adaptive behavior.



INTRODUCTION

Constitutional dynamic chemistry (CDC)¹ is predicated on the use of reversible interconnections to generate structurally diverse dynamic libraries of molecular^{1–3} or supramolecular entities from discrete sets of molecular subunits. The available subunits, and their interconnection capacities, define a virtual combinatorial library (VCL)^{3a,b,d} of constituents, formed by all possible subunit permutations, with the actual distribution of entities present at equilibrium corresponding to the global energy minimum of the system. A key aspect of such thermodynamically controlled networks is their sensitivity to physical and/or chemical factors that perturb equilibria underlying subunit association, thus shifting the observed distributions of higher-order entities. In principle, a given external stimulus may cause a directed reconfiguration of subunits, so that previously unexpressed members of the VCL become dominant at the expense of those initially formed. Establishing control over such dynamic assembly/disassembly processes has become an overarching goal relating to the design of chemical systems of increasing complexity,^{1,4} with recent applications in the areas of nanostructure fabrication,⁵ the development of adaptive materials,^{6,7} and the identification of biologically active compounds in the context of dynamic combinatorial chemistry.^{2,3b,3e,4c}

The realization of CDC in the field of polymer science has engendered interest in dynamic polymers, or dynamers,^{6b,c} as stimulus-responsive materials.^{6,7} Dynamers are defined as polymeric species comprised of monomer units connected by either noncovalent or reversible covalent interconnections.^{6b,c} The lability of the intermonomer linkages affords such systems the possibility of undergoing effector-driven monomer exchange and/or chain elongation/shortening processes (Scheme 1A), which can in turn modulate the chemical and materials properties exhibited by the VCL. Indeed, an eclectic range of polymeric entities possesses such characteristics, including supramolecular polymers,^{5c,6e,6g} some coordination⁸ and condensation⁷ polymers, cylindrical surfactant assemblies,⁹ and certain biomacromolecular aggregates.¹⁰ Poly(azomethine)s,⁷ formed by polycondensation of dicarbonyl and diamino (including di(hydrazino) and di-(alkoxyamino)) containing monomers, represent a particularly important class of covalent dynamers, in view of their synthetic accessibility and controllable dynamic character. The exchange of azomethine (C=N) moieties with alternate amino groups is subject to Bronsted or Lewis acid catalysis¹¹ and may therefore be controlled through the adjustment of pH or catalyst

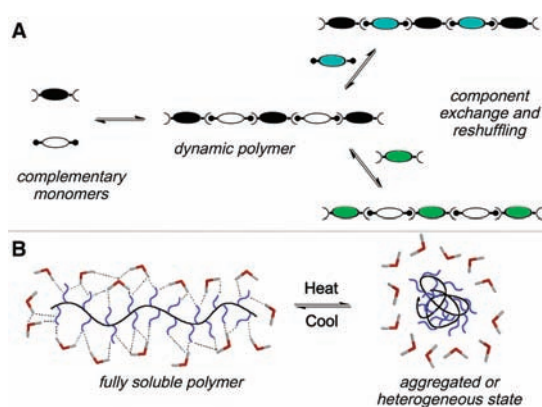
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concentration. Additionally, the intimate relationship between the covalent structure of a polymer and its associated properties implies that adaptive behavior in dynamic covalent systems may be dramatic. Molecular-level reorganization of dynamic azomethine-based networks has been achieved in response to a variety of factors (including H^+ ,^{7f,12} temperature,¹² metal ions,^{7h,12,13} electric fields,¹⁴ hydrogelation,¹⁵ biological agents,^{2,3b,3e,7d} and macromolecular architecture^{7i,16}) and has been shown to modulate the optical,^{7b,e,f,h} rheological,^{7g} and mechanical^{7c} characteristics of poly(azomethine)s, both in solution and in neat films.

Stimulus-responsive behavior is also an important aspect of conventional (constitutionally static) polymer chemistry.¹⁷ So-called “smart” polymers undergo reversible conformational and aggregation-state transitions in solution in response to environmental triggers (i.e., pH, temperature, ionic strength) that generally affect polymer–solvent interactions. In particular, thermoresponsive water-soluble polymers¹⁸ (TWP) have been widely studied in view of potential biological and environmental applications. As smart materials, TWP also benefit from the complete reversibility of thermal stimuli as well as the ease and precision with which temperature can be controlled. TWP are

Scheme 1. Stimulus-Responsive Behavior of Constitutionally Dynamic and Thermoresponsive Polymers^a



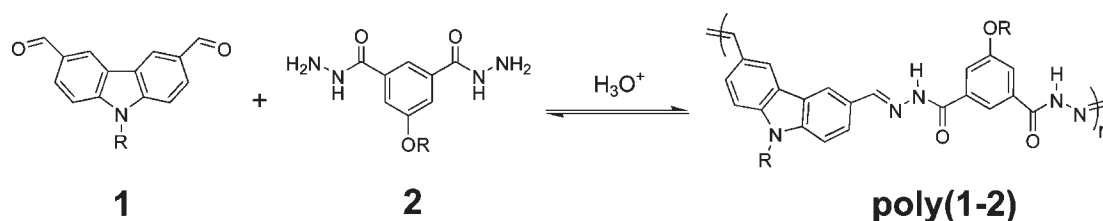
^a (A) Dynamic polymers (dynamers)^{6b,c} may be formed through the reversible interconnection of complementary monomeric subunits. Such polymeric entities may exhibit compositional changes in response to external stimuli that perturb the equilibria underlying monomer interconnection. (B) Thermoresponsive water-soluble polymers^{17,18} (TWP) undergo reversible conformational and/or aggregation-state transitions with temperature. TWP usually consist of a hydrophobic main-chain (black), on which are grafted a number of hydrogen bonding groups (blue) that organize nearby solvent molecules. With increasing temperature, unfavorable entropy of solvation causes a weakening of polymer–solvent interactions and eventual hydrophobic collapse.

typically nonionic, with a subtle balance of hydrophobicity and hydrogen-bonding capacity along the main chain. As temperature increases, entropically unfavorable polymer–solvent hydrogen bonds are disrupted, leading to hydrophobic collapse (Scheme 1B) and often to an abrupt and reversible precipitation of the polymer at the lower critical solution temperature (LCST).¹⁹ The most thoroughly investigated TWP include poly(isopropylacrylamide),²⁰ poly(ethylene glycol),²¹ and elastin-like peptides²² (rich in the sequence Val-Pro-Gly-Xaa-Gly, where Xaa is a variable residue). In recent years, these and other TWP have been integrated into numerous technologies, including hyperthermic drug delivery,^{18c,23} bioengineering,²⁴ the regulation of nanoscale self-assembly,²⁵ and the construction of hydrogel-based thermo-²⁶ and chemomechanical devices.²⁷

Rationale. Our interest in CDC as a means of engineering novel stimulus-responsive materials led us to pursue the development of thermoresponsive poly(azomethine) dynamers. Such systems may be capable of responding to thermal stimuli both through compositional reorganization of the main chain (Scheme 1A) and through changes in polymer conformation and/or aggregation state (Scheme 1B). Although these two types of stimulus-responsive behaviors have separate origins, it is expected that they will operate jointly in influencing the structure and properties of the system.

Previously, we reported the synthesis, structural characterization, and constitutionally dynamic behavior of a series of water-soluble, aromatic poly(acylhydrazones) with appended hexa(ethylene glycol) solubilizing groups.²⁸ In particular, monomers **1** and **2** were reacted to give the poly(acylhydrazone) poly(**1–2**) (Scheme 2), which was determined to assume a helical conformation in aqueous solution that minimizes solvent-exposed hydrophobic surface area. The proposed helical secondary structure, which is supported by small-angle neutron scattering (SANS), size-exclusion chromatography/multiangle laser light scattering (SEC/MALLS), and transmission electron microscopy (TEM) data, constitutes a water-soluble, organic nanotube, with an inner diameter of approximately 1.3 nm. In addition, control was demonstrated over the CDC of the system by exploiting the pH dependence of acylhydrazone reactivity. Under acidic conditions (pH 2.0), the acylhydrazone linkages of poly(**1–2**) can form, break, and exchange reversibly,^{11c,29} causing the molecular weight distribution to respond to changes in concentration, whereas above pH ~ 7 the acylhydrazone bonds are effectively static, and poly(**1–2**) behaves as a conventional polymer. The primary structure of poly(**1–2**) resembles those of several known TWP,²¹ as it contains a hydrophobic aromatic core, onto which are grafted oligo(ethylene glycol) hydrophiles, and in fact, LCST behavior was noted during its original preparation. Herein, we explore the effects of thermal stimuli on poly(**1–2**) at

Scheme 2. Acid-Catalyzed Dynamic Polymerization of Di(aldehyde) **1** and Di(acylhydrazine) **2** to Give the Polyacylhydrazone poly(**1–2**)^a



^a R = $(CH_2CH_2O)_6Me$.

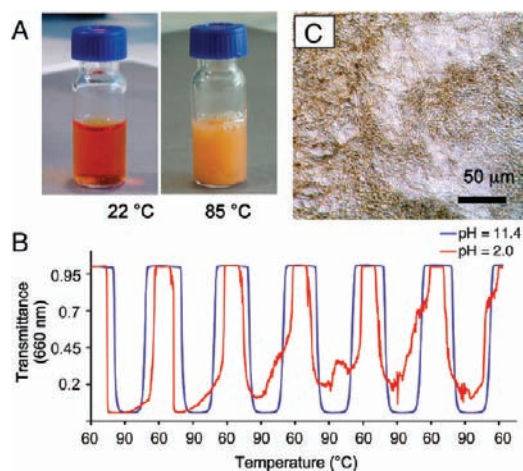


Figure 1. (A) Photographs of aqueous solutions of **poly(1–2)** below and above the lower critical solution temperature (LCST). (B) Turbidimetry diagrams at 660 nm for aqueous solutions of **poly(1–2)** ($[1]_0 = [2]_0 = 1.0$ mM) under acidic and basic conditions as a function of heat/cool cycles (rate = $1\text{ }^\circ\text{C}\cdot\text{s}^{-1}$). (C) Optical micrograph of precipitate irreversibly formed by heating a solution of **poly(1–2)** ($[1]_0 = [2]_0 = 5.0$ mM, pH = 2.0) to $90\text{ }^\circ\text{C}$ for 16 h.

both the molecular and supramolecular levels and report the *reversible growth* of the dynamic polymer with increased temperature. When the monomer units of **poly(1–2)** react under thermodynamic control, the system may respond reversibly to changes in temperature through both compositional and conformational changes, thus establishing an interrelationship between molecular and supramolecular polymer structure that may be regulated by the addition and removal of heat.

RESULTS

The thermal response of **poly(1–2)** was investigated by different physicochemical techniques to establish the ability of these entities to adapt to temperature changes by constitutional variation.

Turbidimetry. The hexa(ethylene glycol) moieties appended to **1** and **2** provide thermal sensitivity to **poly(1–2)** in aqueous solution. Such groups generally become more hydrophobic at elevated temperatures because of negative dissolution entropies, which reflect their tendency to organize solvating water molecules through hydrogen bonding.¹⁹ As anticipated on this basis, **poly(1–2)** was found to display qualitatively reversible LCS behavior (Figure 1A). Heating **poly(1–2)** in aqueous solution to near reflux temperatures causes the sample to undergo microphase separation, and cooling the sample to room temperature results in the rapid redissolution of the polymer. At concentrations greater than approximately $[1]_0 = [2]_0 = 5$ mM, thermally induced microphase separation causes a noticeable increase in the viscosity of the sample upon cooling to the homogeneous state. The turbidimetry profiles shown in Figure 1B reveal that the LCS behavior is markedly pH-dependent. At pH 11.5, a sharp and reversible phase transition is observed at $81\text{ }^\circ\text{C}$ with significant hysteresis. Under acidic conditions (pH 2), on the other hand, a sharp precipitation is initially seen at $76\text{ }^\circ\text{C}$, with further changes becoming broad and erratic with repeated thermal cycling. The irregularity of the low-pH response after several transitions reflects the formation of visible particles from the microphase-separated state that begin to settle out of solution at high temperature but redissolve below the LCST. Maintaining

such a sample above the LCST for prolonged periods of time ($90\text{ }^\circ\text{C}$ for 16 h) causes the polymer to irreversibly precipitate from solution as a fibrous material (Figure 1C). In contrast, no discrete particles are observable by eye in the microphase-separated state in neutral to alkaline solution, and the LCS behavior remains completely reversible with extended heating under such conditions.

In light of the above-mentioned pH dependence of acylhydrazone reaction kinetics,²⁹ the irregularity of the low-pH turbidimetry profile can be attributed to the dynamic state of **poly(1–2)**, which renders the covalent structure of the system sensitive to external factors. It is likely that the transition from a homogeneous solution to a microphase-separated state represents a compelling stimulus, capable of effecting significant constitutional modification of the system. Accordingly, the erratic low-pH LCS behavior becomes less severe if the sample is held above the transition temperature for shorter periods of time, as the constitutional modification likely proceeds to a lesser extent in this case. Further, the effect appears to be reversible over longer time periods, as an acidic solution of **poly(1–2)**, that has been made to exhibit broad transitions through heating, shows regenerated LCS behavior after standing at room temperature for five days. These observations imply that under acidic conditions thermally induced microphase separation causes a relatively slow modulation of the covalent structure of **poly(1–2)**, which initially manifests as a broadening of turbidimetry profile and, upon further heating, by the formation of insoluble, fibrous material, and very large objects up to the macroscale.

Size Exclusion Chromatography/Multiangle Laser Light Scattering (SEC/MALLS). The response of **poly(1–2)** to thermal stimuli was further investigated by SEC/MALLS. A solution of **poly(1–2)** was prepared by reacting **1** (6.1 mM) and **2** (5.9 mM) in H_2O (pH 2.0) for 48 h at ambient temperature, and an aliquot was removed and analyzed to give a weight-averaged molecular weight (M_w) of 310 kDa (Figure 2, trace A). Immediately after heating the solution above the LCST (to $85\text{ }^\circ\text{C}$) for 4 h, the M_w was found to have increased by over an order of magnitude ($M_w \sim 3800$ kDa; Figure 2, trace B), with increased polydispersity and a poor correlation between injected and calculated mass. This suggests that in this case the M_w of this sample is too large for accurate determination and that some material may have been retained on the SEC columns. The solution was then allowed to stand at room temperature for 20 h, after which the M_w had decreased to very near the initial value (460 kDa; Figure 2, trace C). Under basic conditions, this dramatic, thermally induced M_w increase is not observed, as heating **poly(1–2)** above the LCST for 4 h at pH 11.4 causes no appreciable change in M_w . It is therefore clear that heating aqueous solutions of **poly(1–2)** above the LCST causes a large and reversible increase in M_w under acidic conditions in which the acylhydrazone bonds can break and form reversibly but has little effect under basic conditions, where reaction of acylhydrazone bonds is intrinsically very sluggish.

The influence of temperature on the M_w of **poly(1–2)** under homogeneous conditions (below the LCST) was also examined. Figure 3 shows SEC traces of identical solutions of **poly(1–2)** that were allowed to equilibrate for 3 days at various temperatures. In all cases, the samples remained homogeneous throughout their preparation. A clear increase in retention time with temperature is observed, and with the exception of the highest temperature sample prepared at $63\text{ }^\circ\text{C}$, polydispersities and calculated-injected mass correlations are roughly consistent

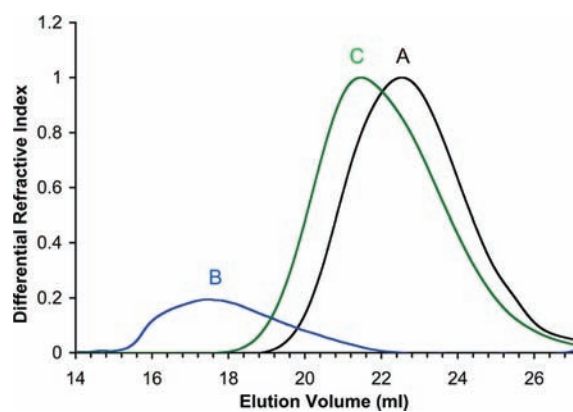


Figure 2. Size exclusion chromatography (SEC) traces of a sample of poly(1–2) prepared by mixing 1 (6.1 mM) and 2 (5.9 mM) in water at pH 2.0 for 48 h (black trace, $M_w = 310 \pm 30$ kDa), immediately after heating the sample above the lower critical solution temperature (to 80 °C) for 4 h (blue trace, $M_w \sim 3800$ kDa) and after allowing the sample to cool to room temperature and stand for 20 h (green trace, $M_w = 460 \pm 50$ kDa). A poor correlation between calculated and injected masses prevents an accurate determination of the high M_w sample (blue trace). M_w values determined by multiangle laser light scattering (MALLS).

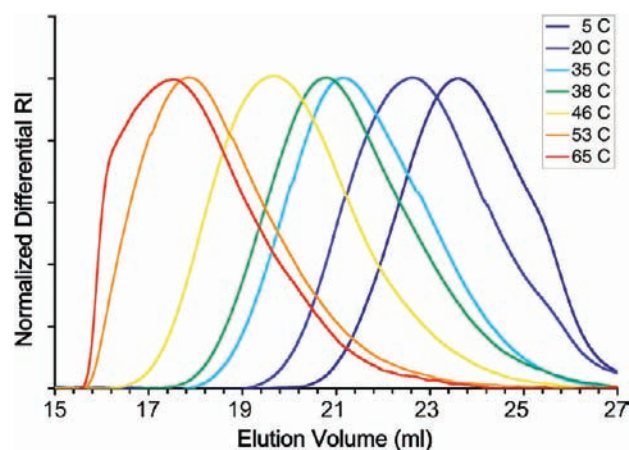


Figure 3. Size exclusion chromatography traces of aqueous solutions of poly(1–2) ($[1]_0 = [2]_0 = 550 \mu\text{M}$, pH = 1.8) equilibrated for 3 days at different temperatures.

among the samples. For the sample prepared at 63 °C, the high molecular weight portion of the peak is truncated, and the material did not pass completely through the SEC columns, which suggests that the M_w is too large for accurate analysis. Figure 4 shows a plot of determined M_w values at various equilibration temperatures for the samples from Figure 3, as well as several control samples, prepared under alkaline conditions. In the dynamic (low-pH) state, a dramatic increase in polymer size with temperature, beginning above around 40 °C, is observed, whereas no change in M_w occurs under alkaline conditions, which further supports the interpretation that thermally induced polymer growth involves acylhydrazone polycondensation. Upon cooling a high- M_w sample that had equilibrated for 3 days at 55 °C under acidic conditions to room temperature, a steady decrease in M_w is observed over time (Figure 5), thus demonstrating the qualitative reversibility of the process.

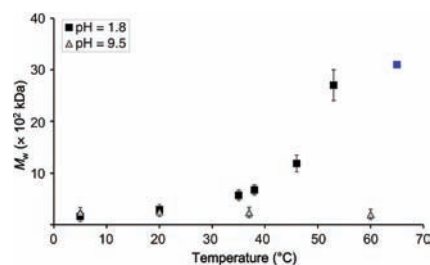


Figure 4. Weight-averaged molecular weight (M_w) determined by SEC/MALLS as a function of temperature for samples of poly(1–2) from Figure 3, as well as identical control samples, adjusted to pH 9.5 prior to temperature adjustment. The M_w of the 65 °C sample (blue square) is too large to be determined accurately and therefore represents a crude estimate.

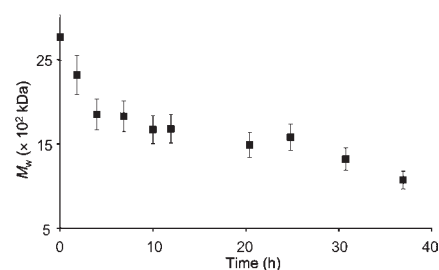


Figure 5. Weight-averaged molecular weight (M_w) determined by SEC-MALLS as a function of time after cooling a sample of poly(1–2) which had equilibrated for 3 days at 55 °C to ambient temperature.

MALLS analysis of fractionated SEC samples revealed details related to the effects of temperature on the structure of poly(1–2). Figure 6 shows variations in radius of gyration (R_g) with molecular mass (m.m.) as determined by SEC-MALLS for samples from Figures 2, 4, and 5. The observed linear relationship is consistent with the classical description of R_g for rodlike objects³⁰ and indicates that the linear density (mass per unit length) of poly(1–2) is $720 \pm 60 \text{ Da} \cdot \text{\AA}^{-1}$. This value closely agrees with those both previously measured by SEC-MALLS and small-angle neutron scattering and predicted by a molecular model of a helically folded, single chain of poly(1–2).^{28b} The colinearity of the data in Figure 6 indicates that the basic morphology is unchanged upon heating and cooling (lateral aggregation of two polymer chains would reduce the slope of R_g vs m.m. by a factor of 2), implying that the resultant changes in M_w correspond to the lengthening and shortening of the polymer along the rod axis. The single distinctly nonlinear curve in Figure 6 is the high-molecular weight sample from Figure 2, which is the only sample that was analyzed immediately after heating above the LCST; all other samples had been homogeneous for an extended period (20 h) prior to analysis or had remained so throughout their preparation. The nonlinearity of this outlier indicates an alternative solution state morphology, suggesting that above the LCST poly(1–2) undergoes a different mode of growth relative to the elongation of the well-characterized²⁸ rods that occurs with heating under homogeneous conditions.

Transmission Electron Microscopy (TEM). The system was further investigated by TEM, which allows for the direct visualization of thermally induced changes in polymer structure. Figure 7a shows a micrograph of a 1.1 mM sample of poly(1–2)

that had been prepared under acidic conditions at room temperature. In accordance with previous studies,²⁸ negatively stained rodlike structures with a constant diameter of around 5 nm and lengths ranging from 50 to 80 nm are observed (see also Supporting Information). Aggregates of two or more rods are also visible. The physical dimensions of these filaments agree with a structural model previously proposed²⁸ on the basis of SANS, TEM, and SEC/MALLS measurements, and the objects have therefore been interpreted as being single polymer entities. After heating the sample to 60 °C for 4 h, a clear elongation of the rods is apparent (Figure 7b), with many growing to lengths of more than 200 nm, although the diameter remains identical to that seen prior to heating, which indicates that the basic polymer

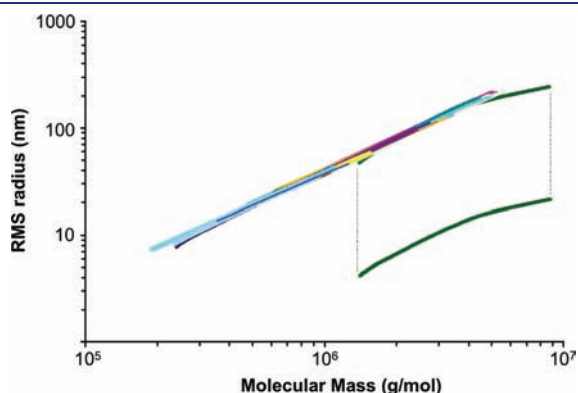


Figure 6. Radius of gyration vs molecular mass relationships determined by MALLS analysis of SEC fractions for samples from Figures 2, 4, and 5. A linear relationship is indicative of rodlike objects,³⁰ and the slope is related to the linear density of the rods. The nonlinear green curve corresponds to the high M_w sample from Figure 2 and has been offset for clarity.

architecture is maintained. Upon standing at room temperature, the gradual shortening of the rods occurs over time (Figure 7c), with qualitative reversion to the initial state (prior to heating) after 1 week (Figure 7d). With the onset of phase transition above the LCST, a dramatic deviation from the prevailing morphology is observed, as the rods become intertwined to form larger fibers that exhibit multiple branching points and achieve micrometer lengths (Figures 7e,f). A fundamental structural change upon heating above the LCST is also reflected by the anomalous nonlinear variation of R_g with molecular mass for a similarly prepared sample offset in Figure 6. These observations corroborate the SEC/MALLS data and unambiguously demonstrate the reversible, thermally induced growth of **poly(1–2)**. More precisely, when the system is heated while maintaining solution homogeneity ($T < \text{LCST}$), smooth and reversible polymer growth occurs along the rod axis. In contrast, thermally induced microphase separation causes relatively uncontrolled growth, coupled with aggregation, which leads to the assembly of large polymer bundles (Figure 7F) and ultimately the precipitation of fibrous material (Figure 1c).

DISCUSSION

The growth of **poly(1–2)** with temperature may appear counterintuitive in view of the fact that dynamic polymers usually tend to break apart at elevated temperatures to minimize entropic costs associated with polymerization.³¹ Known exceptions include certain reversible ring-opening processes, such as the formation of molten sulfur from S_8 rings,³² as well as some coordination³³ and supramolecular polymerizations³⁴ of ditopic subunits capable of forming macrocycles. In each of these cases, the favorable entropy of ring fragmentation apparently overwhelms the entropic penalty of polymerization, rendering the overall process endoentropic. Along different lines, the thermally

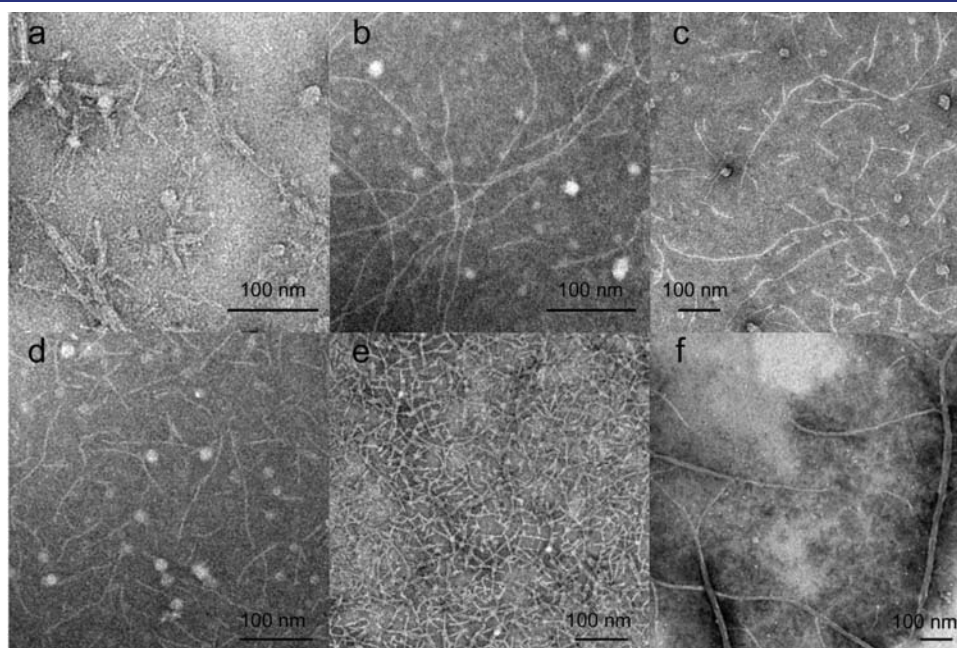
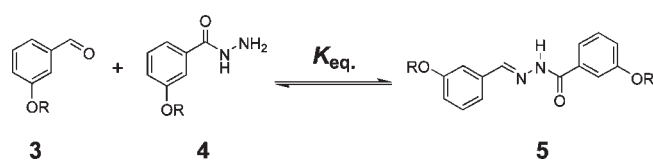


Figure 7. Thermally induced growth of **poly(1–2)** as observed by transmission electron microscopy. (a) Image of a solution of **poly(1–2)** ($[1]_0 = [2]_0 = 1.1 \text{ mM}$, $\text{pH} = 1.9$) after equilibration for 3 days at 20 °C. (b) Image of sample from (a) immediately after heating to 60 °C for 4 h. (c) Image of sample from (b) after standing at 20 °C for 4 h. (d) Image of sample from (b) after standing at 20 °C for 1 week. (e) Image of a solution of **poly(1–2)** ($[1]_0 = [2]_0 = 6.6 \text{ mM}$, $\text{pH} = 2.0$) after equilibration for 3 days at 20 °C. (f) Image of sample from (e) taken immediately after heating to 85 °C for 4.5 h.

Scheme 3. Acid-Catalyzed Dimerization of an Acylhydrazone Model System^a

^a R = (CH₂CH₂O)₆Me.

induced elongation of cationic wormlike micelles has been reported in the presence of particular counterions and over specific concentration regimes.³⁵ This anomalous behavior was explained in terms of the temperature dependence on counterion binding to the charged micelle surface, which influences the effective surface charge and morphology of the micelles. Neither of these known mechanisms for thermally induced dynamic polymer growth are likely to apply in the present case, as **poly(1–2)** is nonionic and there is no evidence of macrocyclic species.

The simplest explanation of the results presented herein would be that the growth of **poly(1–2)** with temperature is a consequence of the intrinsic thermodynamic parameters governing acylhydrazone condensation. If the equilibrium coupling of acylhydrazine (hydrazide) and aldehyde termini to give an acylhydrazone linkage proceeds with a positive (favorable) entropy, the $T\Delta S_{\text{eq}}$ contribution to the overall Gibbs free energy will become increasingly negative (favorable) with temperature, which, assuming a negligible change in heat capacity, would stabilize intermonomer bonds and drive the system toward an increased degree of polymerization. Intuitively, a positive reaction entropy seems unlikely, however, as the acylhydrazone product will probably possess less conformational freedom than the reactants, and the translational entropies of reactants and products should be similar. Nevertheless, this hypothesis was tested through the study of the monomer–dimer model system shown in Scheme 3 by means of variable-temperature ¹H NMR spectroscopy. While the ¹H NMR spectrum of **poly(1–2)** is extremely broad²⁸ (as expected for rapid T2 relaxation characteristic of macromolecules),³⁶ the equilibrium composition of the model system is readily estimated from the integrated signal intensities of the singlets at approximately 9.9 and 8.4 ppm, which arise from the aldehyde and hydrazone CH protons of **3** and **5**, respectively. As shown in Figure 8A, a pronounced shift toward hydrolysis products with increasing temperature is evident, and van't Hoff analysis of equilibrium ratios determined from 25 to 70 °C at 5 °C intervals (Figure 8B) confirms that the reaction is enthalpy driven, with $\Delta H_{\text{eq}} = -8.2 \pm 0.2 \text{ kcal}\cdot\text{mol}^{-1}$ and $\Delta S_{\text{eq}} = -11.1 \pm 0.4 \text{ cal}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$. Purely on this basis, the degree of polymerization of **poly(1–2)** would be expected to diminish with temperature, as is commonly observed for dynamic polymers. Therefore, the intrinsic thermodynamic characteristics of the acylhydrazone condensation can be ruled out as the major factor underlying the thermally induced growth of **poly(1–2)**.

In light of the inverse temperature–solubility relationship of **poly(1–2)** and the tendency of the acylhydrazone linkage to fragment at elevated temperatures, an interplay between the temperature stimulus-responsiveness of **poly(1–2)** at the molecular and supramolecular levels can be evoked to explain the observed behavior. By the mechanism outlined in Scheme 4, elevated temperature would initially cause polymer aggregation (supramolecular response) by virtue of increased hydrophobicity

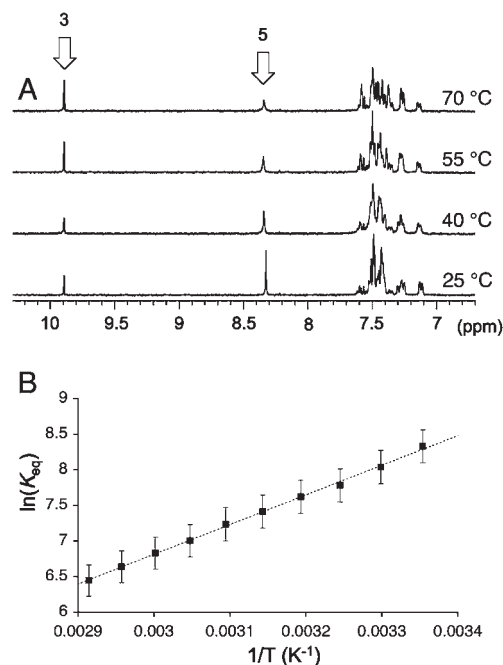
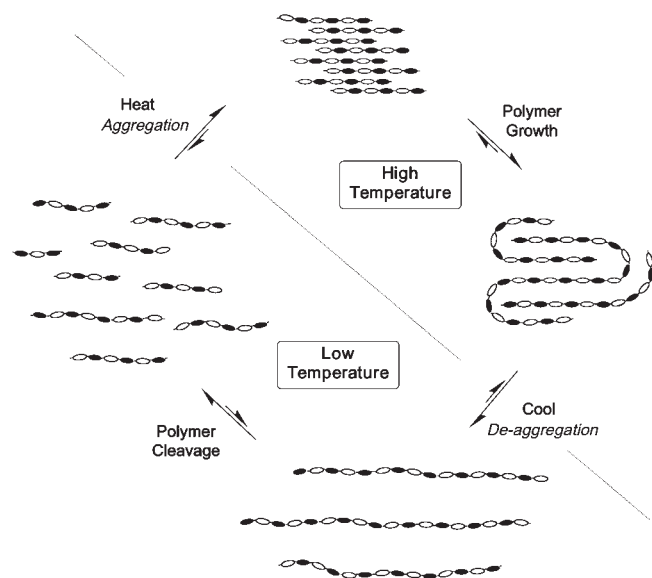


Figure 8. van't Hoff analysis of the acylhydrazone condensation from Scheme 3. (A) The aromatic region of the ¹H NMR spectrum of a solution of **3** ([**3**]₀ = 2.0 mM) and **4** ([**4**]₀ = 2.3 mM) in D₂O (5 mM phosphate buffer, pD = 2.0) at representative temperatures. The singlet at ~9.9 ppm corresponds to the signal for the aldehyde CH proton of **3**, and the singlet at ~8.4 ppm is assigned as the hydrazone CH resonance of **5**. (B) A van't Hoff plot for the sample from A, in which the apparent equilibrium constant for the formation of **5** (K_{eq}) was calculated from the integrated signal intensities of the aldehyde and hydrazone CH resonances of **3** and **5**, respectively. The determined thermodynamic parameters are: $\Delta H_{\text{eq}} = -8.2 \pm 0.2 \text{ kcal}\cdot\text{mol}^{-1}$ and $\Delta S_{\text{eq}} = -11.1 \pm 0.4 \text{ cal}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$.

imparted by the hexaglyme moieties. This aggregation process amounts to an increase in the local concentration of **poly(1–2)**, which promotes further polymerization (molecular response) through dynamic acylhydrazone condensation under Le Chatelier's principle. When the system remains homogeneous (heating below LCST), the interpolymer associations are imagined to be relatively fleeting and mild, resulting in smooth polymer growth along the rod axis, with preservation of the basic rodlike morphology. On the other hand, the onset of a microphase-separated state above the LCST represents a much more severe supramolecular stimulus, causing rapid and uncontrolled polymer aggregation and growth with the formation of large bundles of intertwined rods as observed by TEM. Cooling such elongated polymer assemblies causes a return toward the initial state, as the increased hydrophilicity of the polymer at lower temperatures will promote its deaggregation, followed by covalent reorganization (through preferential acylhydrazone hydrolysis) to accommodate the decrease in local polymer concentration. It has previously been suggested that thermally induced phase transitions will lead to large alterations in the degree of polymerization of dynamic polymers and should therefore be included among the mechanisms of dynamic polymerization.³⁷

The kinetics of equilibration within the macromolecular system are found to be dramatically slower than those observed for the small-molecule system of Scheme 3. At the molecular level, this effect is attributed to the proposed helical conformation of

Scheme 4. Proposed Mechanism for the Thermally Induced Growth of poly(1-2) under Conditions of Reversible Acylhydrazone Condensation^a



^a Heating an equilibrated solution causes polymer aggregation, which in turn leads to polymer growth in regions of increased local concentration. Cooling the resultant elongated polymer assemblies breaks up the aggregation, causing the system to return toward the initial state.

poly(1-2),²⁸ in which the folded aromatic backbone is thought to shield the acylhydrazone linkages from bulk solution. The lack of completely reversible M_w changes in response to thermal stimuli may be due to such kinetic effects and/or to minor irreversible side processes including denaturing and aggregation of the polymer. In terms of supramolecular structure, poly(ethylene glycol) has been reported to form kinetically stable aggregates in water,³⁸ and so it is possible that the depolymerization of poly(1-2) upon cooling could be protracted by similar phenomena.

CONCLUSIONS

The present study demonstrates the *thermally induced growth* of a nonionic, water-soluble dynamic polymer (dynamer) under conditions in which the intermonomer acylhydrazone linkages can break and form reversibly. This behavior is unusual, as reversible polymerizations typically shift toward the formation of lower molecular weight species with increased temperature to minimize the entropic costs of polymerization. As evidenced from SEC/MALLS and TEM measurements, heating solutions of poly(1-2) under acidic conditions ($\text{pH} \sim 2.0$) causes a steady increase in M_w with the preservation of the rodlike morphology, up until the LCST is reached. Above this temperature, abrupt microphase separation occurs, and the M_w increases rapidly, with the formation of mesoscopic polymer bundles and, ultimately with prolonged heating, insoluble fibrous material. The qualitative reversibility of polymer growth with heating both above and below the LCST has been demonstrated, and no polymer growth is observed under alkaline conditions, in which the intermonomer bonds are effectively static. In light of the inverse temperature–solubility relationship of poly(1-2) (Figure 1) and van't Hoff analysis indicating the enthalpy-driven nature of acylhydrazone condensation (Figure 8), the anomalous,

thermally induced growth of poly(1-2) is tentatively attributed to a prior aggregation of the polymer, which increases its local concentration and drives the equilibrium system toward the expression of higher molecular weight species.

From a broader perspective, the present results demonstrate the utility of CDC in reversibly influencing the structural and morphological features of chemical systems at the nanoscale and beyond. The use of heat as a controlling effector is especially advantageous in this context, as it is operationally simple, compatible with a wide range of systems, and completely reversible (no byproducts are accumulated). The anomalous polymer growth/degradation behavior represents an *adaptation of a constitutionally dynamic system* in response to a physical stimulus, heat. In addition, it is important to note that the behavior of the present system is as well dependent on the pH of the medium. It therefore is subject to *double control* through two orthogonal agents: heat and protons. On this basis, constitutionally dynamic systems exhibiting a higher level of adaptive behavior and multiple control can be envisioned, which would undergo thermally controlled morphological or component selection processes, thus allowing for species of radically different shape or chemical composition to be reversibly expressed from a given set of constituents under the influence of added or removed heat. Efforts along these lines are presently underway.

ASSOCIATED CONTENT

S Supporting Information. Experimental section detailing syntheses of monomers and experimental methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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REFERENCES

- (1) (a) Lehn, J.-M. *Chem. Soc. Rev.* **2007**, *36*, 151–160. (b) Lehn, J.-M. *Science* **2002**, *295*, 2400–2403. (c) Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4763–4768.
- (2) (a) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652–3711. (b) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898–952.
- (3) (a) Lehn, J. M. *Chem.—Eur. J.* **1999**, *5*, 2455–2463. (b) Huc, I.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2106–2110. (c) Storm, O.; Lüning, U. *Chem.—Eur. J.* **2002**, *8*, 793–798. (d) Hasenknopf, B.; Lehn, J.-M.; Kneisel, B. O.; Baum, G.; Fenske, D. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1838–1840. (e) Hochgürtel, M.; Kroth, F.; Piecha, D.; Hofman, M. W.; Nicolau, C.; Krause, S.; Schaaf, O.; Sonnenmoser, G.; Eliseev, A. V. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 3382–3387. (f) Ramström, O.; Lehn, J.-M. *Nat. Rev. Drug Discovery* **2002**, *1*, 26–36.

- (4) (a) Diederich, F. *Angew. Chem., Int. Ed.* **2007**, *46*, 68–69. (b) Ludlow, R. F.; Otto, S. *Chem. Soc. Rev.* **2008**, *37*, 101–108. (c) Rozenman, M. M.; McNaughton, B. R.; Liu, D. R. *Curr. Opin. Chem. Biol.* **2007**, *11*, 259–268.
- (5) Selected reviews and examples: (a) Meyer, C. D.; Joiner, C. S.; Stoddart, J. F. *Chem. Soc. Rev.* **2007**, *36*, 1705–1723. (b) Sreenivasachary, N.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5938–5943. (c) Todd, E. M.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2007**, *129*, 14534–4535. (d) Harley, C. S.; Elliott, E. L.; Moore, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 4512–4513. (e) Fujita, M.; Tominaga, M.; Hori, A.; Therrien, B. *Acc. Chem. Res.* **2005**, *38*, 371–380. (f) Liu, X.; Warmuth, R. J. *Am. Chem. Soc.* **2006**, *128*, 14120–14127. (g) Reinhoudt, D. N.; Crego-Calama, M. *Science* **2002**, *295*, 2403–2407. (h) Collin, J.-P.; Deiterich-Buchecker, C.; Gaviña, P.; Jimenez-Molero, M. C.; Sauvage, J.-P. *Acc. Chem. Res.* **2001**, *34*, 477–487. (i) Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. *Acc. Chem. Res.* **1995**, *28*, 37–44.
- (6) Selected reviews and examples: (a) Cordier, P.; Tournilac, F.; Soulié-Ziakovic, C.; Leibler, L. *Nature* **2008**, *451*, 977–980. (b) Lehn, J.-M. *Prog. Polym. Sci.* **2005**, *30*, 814–831. (c) Lehn, J.-M. *Aust. J. Chem.* **2010**, *63*, 611–623. (d) Nishinaga, T.; Tanatani, A.; Oh, K.; Moore, J. S. *J. Am. Chem. Soc.* **2002**, *124*, 5934–5935. (e) Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071–4097. (f) Rieth, S.; Baddeley, C.; Badjic, J. D. *Soft Matter* **2007**, *3*, 137–154. (g) Kamplain, J. W.; Bielawski, C. W. *Chem. Commun.* **2006**, 1727–1729. (h) *Supramolecular Polymers*; Ciferri, A., Ed.; Marcel Dekker: New York, 2000. (i) Amamoto, Y.; Higaki, Y.; Matuda, Y.; Otsuka, H.; Takahara, A. *Macromolecules* **2007**, *40*, 1429–1434.
- (7) (a) Ruff, Y.; Lehn, J.-M. *Biopolymers* **2008**, *89*, 486–496. (b) Ono, T.; Fujii, S.; Nobori, T.; Lehn, J.-M. *Chem. Commun.* **2007**, 4360–4362. (c) Ono, T.; Fujii, S.; Nobori, T.; Lehn, J.-M. *Chem. Commun.* **2007**, 46–48. (d) Sreenivasachary, N.; Hickman, D. T.; Sarazin, D.; Lehn, J.-M. *Chem.—Eur. J.* **2006**, *12*, 8581–8588. (e) Skene, W. G.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8270–8275. (f) Giuseppone, N.; Fuks, G.; Lehn, J.-M. *Chem.—Eur. J.* **2006**, *12*, 1723–1735. (g) Kolomiets, E.; Lehn, J.-M. *Chem. Commun.* **2005**, 1519–1521. (h) Giuseppone, N.; Lehn, J.-M. *J. Am. Chem. Soc.* **2004**, *126*, 11448–11449. (i) Zhao, D.; Moore, J. S. *J. Am. Chem. Soc.* **2003**, *125*, 16294–16299. (j) Ruff, Y.; Buhler, E.; Candau, J.-S.; Kesselman, E.; Talmon, Y.; Lehn, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 2573–2584.
- (8) Friese, V. A.; Kurth, D. G. *Coord. Chem. Rev.* **2008**, *252*, 199–211. (b) Schubert, U. S.; Eschbaumer, C. *Angew. Chem., Int. Ed.* **2002**, *41*, 2892–2926.
- (9) Cates, M. E.; Candau, S. J. *J. Phys.—Condens. Matter* **1990**, *2*, 6869–6892.
- (10) (a) Oosawa, F. In *Supramolecular Polymers*; Ciferri, A., Ed.; Marcel Dekker: New York, NY, 2000; pp 643–661. (b) Zhao, D.; Moore, J. S. *Org. Biomol. Chem.* **2003**, *1*, 3471–3491. (c) Klug, A. *Angew. Chem., Int. Ed.* **1983**, *22*, 565–582. (d) Weisel, J. W. *Biophys. J.* **1986**, *50*, 1079–1093. (e) Kim, J.; Liu, Y.; Ahn, J.; Zauscher, S.; Karty, J. M.; Tamanaka, Y.; Craig, S. L. *Adv. Mater.* **2005**, *17*, 1749–1753.
- (11) (a) Giuseppone, N.; Schmitt, J.-L.; Schwartz, E.; Lehn, J.-M. *J. Am. Chem. Soc.* **2005**, *127*, 5528–5539. (b) Polyakov, V. A.; Nelen, M. I.; Nazarpak-Kandlousy, N.; Ryabov, A. D.; Eliseev, A. V. *J. Phys. Org. Chem.* **1999**, *12*, 357–363. (c) Nguyen, R.; Huc, I. *Chem. Commun.* **2003**, 942–943. (d) Leach, B. E.; Leussing, D. L. *J. Am. Chem. Soc.* **1971**, *93*, 3377–3384. (e) Cordes, E. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1962**, *84*, 832–837.
- (12) Giuseppone, N.; Lehn, J.-M. *Chem.—Eur. J.* **2006**, *12*, 1715–1722.
- (13) (a) Giuseppone, N.; Schmitt, J.-L.; Lehn, J.-M. *Angew. Chem., Int. Ed.* **2004**, *43*, 4902–4906. (b) Ulrich, S.; Lehn, J.-M. *Angew. Chem., Int. Ed.* **2008**, *47*, 2240–2243.
- (14) Giuseppone, N.; Lehn, J.-M. *Angew. Chem., Int. Ed.* **2006**, *45*, 4619–4624.
- (15) Buhler, E.; Sreenivasachary, N.; Candau, S.-J.; Lehn, J.-M. *J. Am. Chem. Soc.* **2007**, *129*, 10058–10059.
- (16) (a) Oh, K.; Jeong, K.-S.; Moore, J. S. *Nature* **2001**, *414*, 889–893. (b) Zhao, D.; Moore, J. S. *J. Am. Chem. Soc.* **2002**, *124*, 9996–9997.
- (17) (a) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173–1222. (b) de la Heras Alarón, C.; Pennadam, S.; Alexander, C. *Chem. Soc. Rev.* **2005**, *34*, 276–285. (c) Galaev, I. Y.; Mattiasson, B. *Trends Biotechnol.* **1999**, *17*, 335–340. (d) Bhargava, P.; Tu, Y.; Zheng, J. X.; Xiong, H.; Quirk, R. P.; Cheng, S. Z. D. *J. Am. Chem. Soc.* **2007**, *129*, 832–837.
- (18) (a) Dimitrov, I.; Trzebicka, B.; Müller, A. H. E.; Dworak, A.; Tsvetanov, C. B. *Prog. Polym. Sci.* **2007**, *32*, 1275–1343. (b) Morishima, Y. *Angew. Chem., Int. Ed.* **2007**, *46*, 1370–1372. (c) Schmaljohann, D. *Adv. Drug Delivery Rev.* **2006**, *58*, 1655–1670.
- (19) Smith, G. D.; Bedrov, D. *J. Phys. Chem. B* **2003**, *107*, 3095–3097.
- (20) (a) Zhang, X.-Z.; Xu, X.-D.; Cheng, S.-X.; Zhuo, R.-X. *Soft Matter* **2008**, *4*, 385–391. (b) Haba, Y.; Kojima, C.; Harada, A.; Kono, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 234–237.
- (21) (a) Hua, F.; Jiang, X.; Zhao, B. *Macromolecules* **2006**, *39*, 3476–3479. (b) Aathimaniandan, S. V.; Savariar, E. N.; Thayumanavan, S. J. *Am. Chem. Soc.* **2005**, *127*, 14922–14929. (c) Mitchell, J. D.; Tiddy, G. J. T.; Waring, L.; Bostock, T.; McDonald, M. P. *J. Chem. Soc., Faraday Trans. 1* **1983**, *79*, 975–1000.
- (22) (a) Chilkoti, A.; Christensen, T.; MacKay, J. A. *Curr. Opin. Chem. Biol.* **2006**, *10*, 652–657. (b) Reiersen, H.; Clarke, A. R.; Rees, E. *J. Mol. Biol.* **1998**, *283*, 255–264.
- (23) Chilkoti, A.; Dreher, M. R.; Meyer, D. E.; Raucher, D. *Adv. Drug Delivery Rev.* **2002**, *54*, 613–630.
- (24) Stayton, P. S.; Shimoboji, T.; Long, C.; Chilkoti, A.; Chen, G.; Harris, J. M.; Hoffman, A. S. *Nature* **1995**, *378*, 472–474.
- (25) Nath, N.; Chilkoti, A. *Adv. Matter* **2002**, *14*, 1243–1247.
- (26) (a) Luo, Q.; Mutlu, S.; Gianchandani, Y. B.; Svec, F.; Fréchet, J. M. J. *Electrophoresis* **2003**, *24*, 3694–3702. (b) Yoshida, R.; Uchida, K.; Kaneko, Y.; Saki, K.; Kikuchi, A.; Sakurai, Y.; Okano, T. *Nature* **1995**, *374*, 240–242.
- (27) Imanishi, Y.; Ito, Y. *Pure Appl. Chem.* **1995**, *67*, 2015–2021.
- (28) (a) Folmer-Andersen, J. F.; Lehn, J.-M. *Angew. Chem., Int. Ed.* **2009**, *48*, 7664–7667. (b) Folmer-Andersen, J. F.; Buhler, E.; Candau, S.-J.; Joulie, S.; Schmutz, M.; Lehn, J.-M. *Polym. Int.* **2010**, *59*, 1477–1491.
- (29) (a) Ramström, O.; Lohmann, S.; Bunyapaiboonsri, T.; Lehn, J.-M. *Chem.—Eur. J.* **2004**, *10*, 1711–1715. (b) Ferlan, R. L. E.; Ng, Y.-F.; Cousins, G. R. L.; Redman, J. E.; Sanders, J. K. M. *Tetrahedron* **2002**, *58*, 771–778.
- (30) Benoit, H.; Doty, P. *J. Phys. Chem.* **1953**, *57*, 958–963.
- (31) van der Schoot, P. In *Supramolecular Polymers*, 2nd ed.; Ciferri, A., Ed.; CRC Press: Boca Raton, FL, 2005; pp 77–106.
- (32) (a) Cates, M. E. *Europhys. Lett.* **1987**, *4*, 497–502. (b) Tobolsky, A. V.; Eisenberg, A. *J. Am. Chem. Soc.* **1959**, *81*, 780–780.
- (33) Vermonden, T.; van der Gucht, J.; de Waard, P.; Marcelis, A. T. M.; Besseling, N. A. M.; Sudhölter, E. J. R.; Fler, G. J.; Stuart, M. A. C. *Macromolecules* **2003**, *36*, 7035–7044.
- (34) Folmer, B. J. B.; Sijbesma, R. P.; Meijer, E. W. *J. Am. Chem. Soc.* **2001**, *123*, 2093–2094.
- (35) Kalur, G. C.; Frounfelker, B. D.; Cipriano, B. H.; Norman, A. I.; Raghavan, S. R. *Langmuir* **2005**, *21*, 10998–11004.
- (36) Sanders, J. K. M.; Hunter, B. K. *Modern NMR Spectroscopy, A Guide for Chemists*, 2nd ed.; Oxford University Press: New York, NY, 1993; pp 208–211.
- (37) Ciferri, A. In *Supramolecular Polymers*, 2nd ed.; Ciferri, A., Ed.; CRC Press: Boca Raton, FL, 2005; pp 43–45.
- (38) (a) Duval, M.; Sarazin, D. *Polymer* **2000**, *41*, 2711–2716. (b) Khan, M. S. *J. Appl. Polym. Sci.* **2006**, *102*, 2578–2583.