Recognition-Mediated Unfolding of a Self-Assembled Polymeric Globule

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ABSTRACT: Diaminotriazine-functionalized polystyrene folds into a highly compact micelle-like structure in nonpolar solvents, as established by gel permeation chromatography. Folding of this polymer arises from intramolecular hydrogen bonding between the triazine moieties, allowing the unfolding process to be effected through competitive intermolecular host−guest interactions. Variable-temperature NMR titrations of this polymer with a complementary host were used to quantify the thermodynamics of this unfolding process.

Control of the secondary and tertiary structure of macromolecules is of fundamental importance to the fields of biology and materials science. In biological chemistry, correct foldingof nucleic acids[1] and proteins[2] is a prerequisite for their proper functioning. In synthetic polymers, applications of supramolecular control of polymer architecture have included the formation of polymeric tubules through self-assembly between cyclodextrin units,[3] nanocylinders formed through the shaping of polymer chains with dendrimer fragments,[4] helical structures from achiral polyheterocyclic strands,[5] and planar polymers derived from lipid membranes.[6]

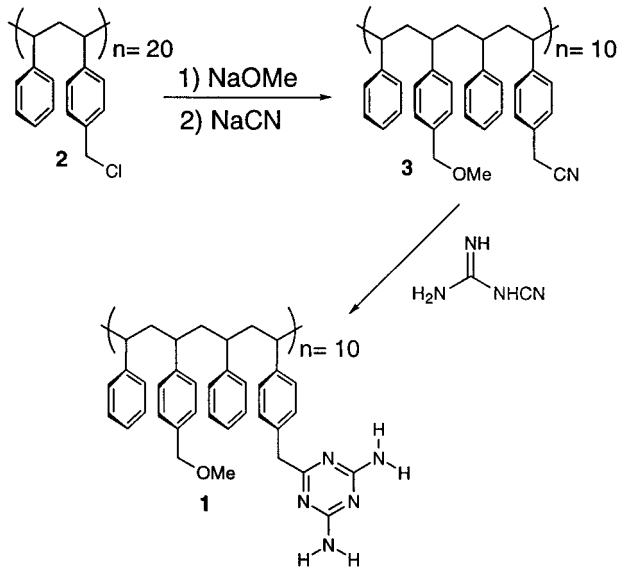
Biological systems can provide important insight for the creation of man-made systems.[7] Globular proteins use a complex balance of solvophobic and intramolecular interactions distributed throughout the tertiary structure of the protein to provide highly compact and specific structures. To provide a model for biopolymer folding, and enhanced versatility for the control of abiological polymer folding,[8] we have synthesized dynamically self-assembled micelle-like[9] polymers. We report here the recognition-mediated folding and unfolding of these polymeric "globules".[10,11]

Diaminotriazine-functionalized polymer **1** provides a system capable of both intra- and intermolecular self-assembly through hydrogen bonding (Scheme 1). Homogeneous dispersion of functionality was obtained by starting with a 1:1 copolymer of styrene and chloromethylstyrene (**2**).[12] This polymer was then partially substituted with sodium methoxide, followed by reaction of the remaining chloromethyl groups with excess sodium cyanide. Reaction of the cyano-functionalized polymer **3** with dicyandiamide then provided polymer **1**. The degree of triazine substitution, and hence solubility and aggregation[13] of polymer **1**, was controlled by the stoichiometry of the methoxide addition.

Preliminary prediction of the structure of polymer **1** was obtained through molecular dynamics calculations on a model polymer, poly(styrene−*p*-(methyldiaminotriazine)styrene (Figure 1).[14] In vacuo calculations predicted a highly compact structure containing multiple intramolecular hydrogen bonds between triazine side chains. This compact structure was quite robust, maintaining integrity at elevated temperatures. In further studies using a continuum solvent treatment, the compact structure was likewise retained.

The presence of the internal hydrogen bonding required for micelle-like folding was experimentally es-

Scheme 1. Synthesis of Triazine-Functionalized Random Copolymer 1



tablished using infrared spectroscopy. The IR spectrum ($CHCl_3$) of polymer **1** relative to monomeric diaminotriazine **4** (Figure 4) exhibited both substantial broadening of the N−H region and shifting of these absorptions to lower wavenumber (Figure 2), indicative of hydrogen bonding.[15] Dilution IR studies of polymer **1** showed no effect of concentration on peak shift and broadening, demonstrating that this behavior arises from intramolecular hydrogen bonds, rather than intermolecular aggregation.

Direct confirmation of the compact micellar folding behavior of polymer **1** in noncompetitive solvents was obtained through gel permeation chromatography (GPC) experiments in $CHCl_3$ (Figure 3).[16] Polymer **1** eluted considerably later than control polymer **3**,[17] indicative of a much smaller radius of gyration (r_g) for polymer **1**.[18] The relative r_g's of these two polymers can be estimated using the relationship

$$\frac{r_g^{\text{polymer3}}}{r_g^{\text{polymer1}}} = \frac{M_{\text{polymer3}}^{a+1}}{M_{\text{polymer1}}^{a+1}} \qquad (1)$$

where M represents the molecular weight of the polystyrene standard at the center of the respective GPC

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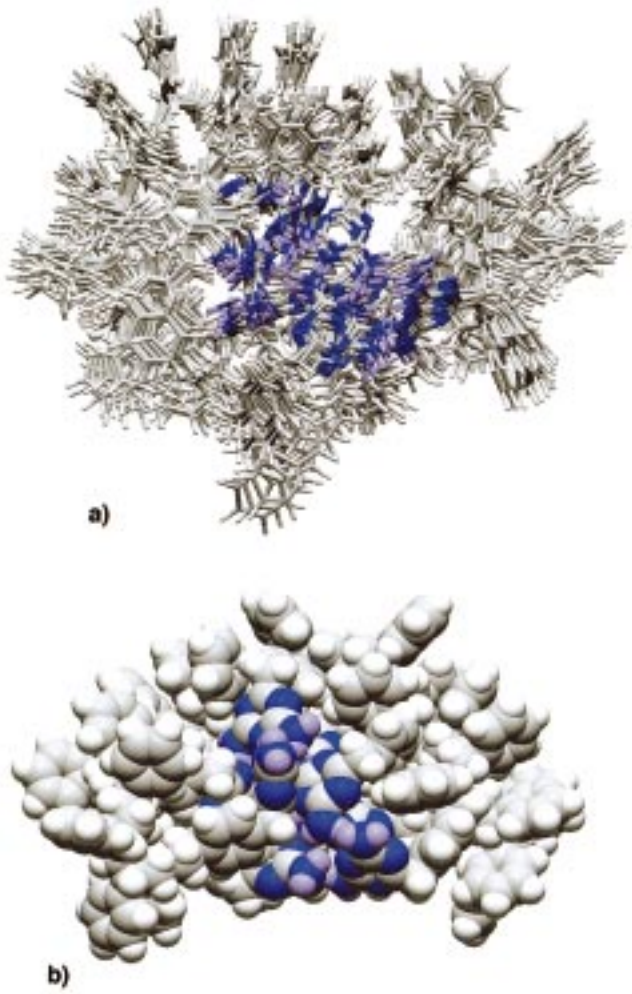


Figure 1. Micelle-like structure predicted (Amber force field) for atactic polystyrene (40 monomer units total) with methylenediaminotriazine substitution at every fourth carbon. (a) Sampled structures from a final 20 ps dynamics simulation (300 K, $CHCl_3$). (b) Sampled structure during dynamics simulation.

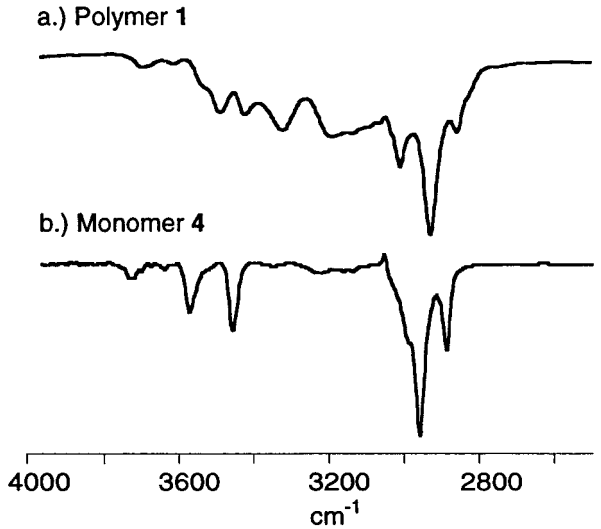


Figure 2. Infrared spectra of (a) polymer **1** (10^{-3} M) and (b) monomer **4** (10^{-3} M). Spectra were obtained in $CHCl_3$ at 298 K.

peaks for polymers **1** and **3**, and *a* is the viscometric exponent (0.73 for polystyrene in $CHCl_3$[19]). From this equation, we can establish a ∼*5.6-fold* reduction in radius of gyration for the triazine-functionalized polymer **1** relative to the nonfolded cyano-polymer **3**,[20] fully consistent with the expected highly compact structure. Intriguingly, the GPC trace for polymer **1** shows multiple peaks, indicating the possibility of specific folded conformations.

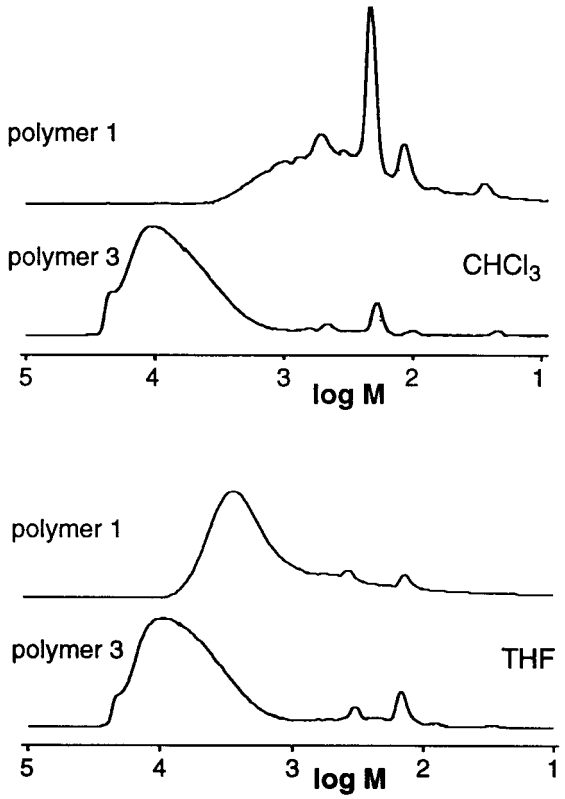


Figure 3. GPC chromatograms for polymer **1** and control polymer **3** in $CHCl_3$ and THF. Log *M* refers to the M_n of polystyrene calibration standards run in each of the solvents. The peaks at ∼200 represent minor oligomeric impurities.[22]

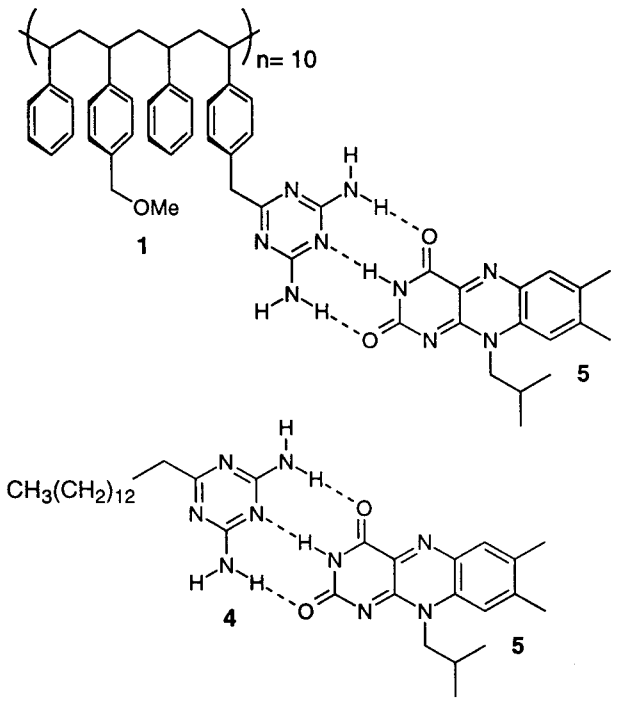


Figure 4. Polymer **1**−flavin **5** and monomer **4**−flavin **5** complexes.

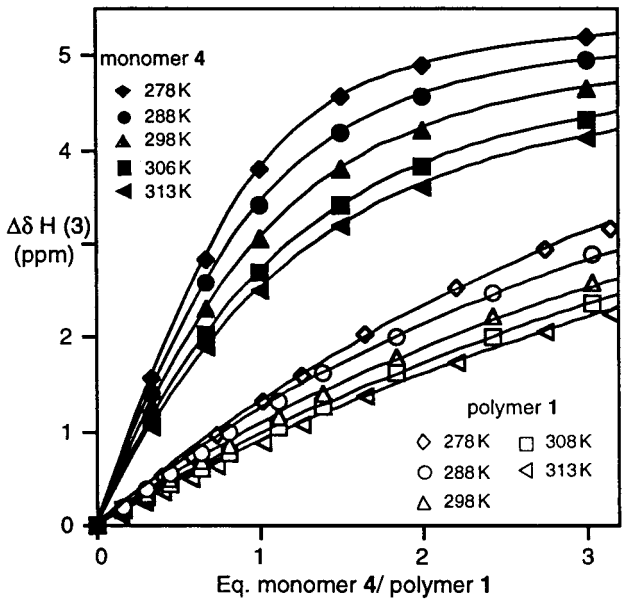


Figure 5. Chemical shift changes of flavin **5** H(3) upon addition of monomer **4** (filled symbols) and polymer **1** (open symbols). Lines represent curve fits to the 1:1 binding isotherms.

Table 1. Binding Constants of Flavin 5 with Polymer 1 and Monomer 4 in $CDCl_3$

T (K)	K_a(**4** + **5**) (M^{-1})[a]	K_a(**1** + **5**) (M^{-1})[a]
278	1140	51
283	900	
288	710	44
293	580	
298	480	36
303	390	
306		35
308	330	
313	270	31

[a] Uncertainties (from standard errors) <10%.

Further evidence that folding of polymer **1** arises from hydrogen bonding came from parallel GPC experiments performed in THF, a competitive hydrogen-bond-accepting solvent. As expected, the elution behavior of non-hydrogen-bonding polymer **3** was essentially identical in the two solvents. In contrast, elution times for polymer **1** were considerably less in THF relative to $CHCl_3$, as expected for the increase in r_g arising from solvent-mediated unfolding. The slightly later elution times observed for polymer **1** relative to polymer **3** in THF indicate that some intramolecular hydrogen bonding still occurs in polymer **1**.

Implicit in the bimolecular recognition of intramolecularly associated polymer **1** is a decrease in the efficiency of *inter*molecular binding arising from the requirement for polymer unfolding prior to the recognition event. This decrease in binding efficiency was established through ^{1}H NMR titration experiments: we observed that polymer **1** binds flavin **5** with an association constant (K_a) of 35.9 M^{-1} ($CDCl_3$),[22−24] more than an order of magnitude less than monomer **4** (476 M^{-1}) (Figure 4).[25] Since the recognition elements of these two systems are electronically identical, the low binding constant of polymer **1**−flavin **5** dyad can be attributed to competitive intramolecular association. As a result, recognition of flavin **5** by polymer **1** requires the unfolding of the polymer tertiary structure.

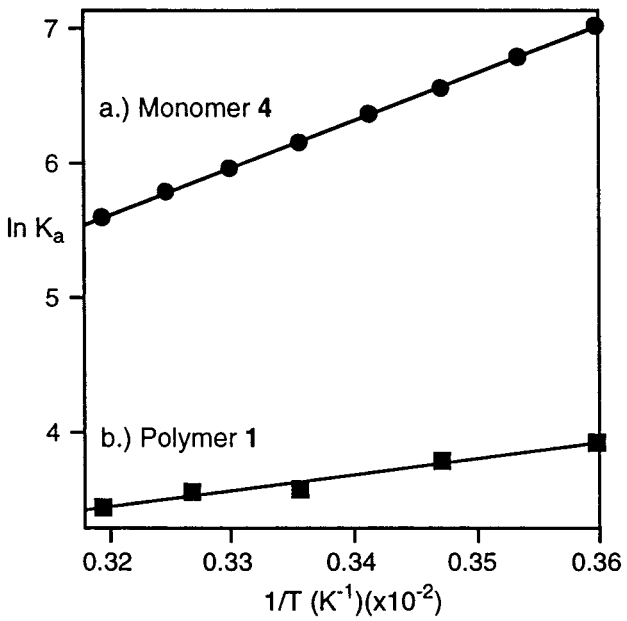


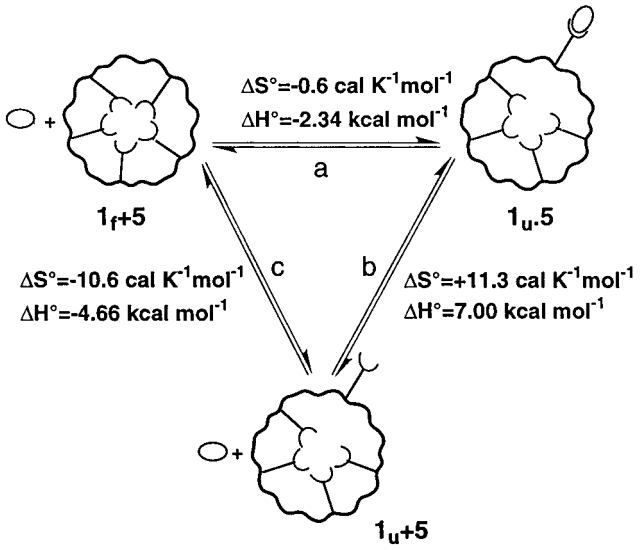
Figure 6. Arrhenius plot of the complexation data resulting from variable-temperature ^{1}H NMR titration experiments of (a) monomer **4** and (b) polymer **1** with flavin **5**.

To quantify the thermodynamics of folding and recognition-mediated unfolding, variable-temperature ^{1}H NMR titrations were performed between flavin **5** and polymer **1** as well as monomer **4** (Figure 5, Table 1). An Arrhenius plot of the resulting complexation data (Figure 6) revealed linear relationships in both cases. As expected, binding of flavin **5** with monomer **4** possessed a strong temperature dependence arising from the loss of translational entropy associated with the recognition process. In contrast, the polymer **1**−flavin **5** binding event showed an extremely weak temperature dependence over the same range.

The extremely weak temperature dependence observed in the complexation of flavin **5** by polymer **1** arises from the competing entropic parameters of intra- and intermolecular self-assembly. To gain further insight into the entropic balance achieved by this system, thermodynamic values obtained from Figure 6 were used to provide a cycle for the recognition-controlled micellar folding/unfolding process (Scheme 2). In this cycle, process a is the experimentally determined binding of flavin **5** to polymer **1** (**1**$_f$), encompassing both the polymer unfolding and the recognition process. Process b represents the binding of flavin **5** to the unfolded polymer (**1**$_u$) and was determined using the thermodynamic values obtained from the isosteric and isoelectronic monomer **4**−flavin **5** dyad. Process c, representing polymer unfolding, was then obtained algebraically from the cycle.

From the thermodynamic cycle, we see that there is an almost perfect balance of entropics for the recognition and unfolding processes, resulting in the very weak temperature dependence for the polymer **1**−flavin **5** complex. The large increase in entropy associated with the unfolding process indicates the complex is quite rigidly self-assembled, consistent with the GPC results in $CHCl_3$. This hypothesis is supported by the relatively large enthalpic cost of unfolding: the enthalpy of the unfolding process (**1**$_u$ → **1**$_f$) is roughly two-thirds that of the recognition process (**1**$_u$ + **5** → **1**$_u$·**5**), where three hydrogen bonds are formed. This indicates that there

Scheme 2. Thermodynamic Cycle for Polymer 1−Flavin 5 Complexation



are approximately two dynamic hydrogen bonds per triazine in the folded state.

In summary, we have synthesized a soluble, diaminotriazine-functionalized polymer. This polymer adopts a micelle-like structure in chloroform solution, driven by the formation of intramolecular hydrogen bonds. This polymer can be unfolded via nonspecific competition from solvents, as well as through specific recognition using a complementary receptor. This unfolding process provides both a method for control of macromolecular tertiary structure and a probe for the energetics of polymer folding. Applications of this system in both fundamental and applied aspects of polymer and biopolymers are underway and will be reported in due course.

Experimental Section

Poly(styrene−*p*-(chloromethyl)styrene, co-PS−CH_2Cl (2). AIBN (4.00 g, 24.4 mmol) was added to a solution of styrene (24.54 g, 235 mmol) and *p*-(chloromethyl)styrene (35.73 g, 234 mmol) in chlorobenzene (200 mL). The reaction mixture was heated at 78 °C for 20 h, followed by cooling to room temperature. The reaction mixture was then added to methanol (800 mL); vigorous mixing resulted in precipitation of a white solid (40.95 g, 68%), which was collected by filtration and washed with methanol. The product was dried in vacuo. GPC: $M_n = 5278$, $M_w = 7729$, PD = 1.465.

Poly(styrene−*p*-(chloromethyl)styrene−*p*-(methoxymethyl)styrene, co-PS−CH_2Cl/ CH_2OCH_3. A solution of polymer (**2**) (1.96 g, 0.37 mmol), sodium methoxide (1.09 mL, 5.7 mmol), and 12 mL of THF was reacted at room temperature for 16 h under an argon atmosphere. The reaction mixture was concentrated in vacuo and precipitated into water. The cream-colored solid product (1.69 g, 87%) was collected by filtration. According to NMR data, 50% of the available sites were converted into methoxide.

Poly(styrene−*p*-(cyanomethyl)styrene−*p*-(methoxymethyl)styrene, co-PS−CH_2CN/ CH_2OCH_3 (3). A solution of co-PS−CH_2Cl/CH_2OCH_3 (1.70 g, 0.325 mmol), sodium cyanide (0.605 g, 12.34 mmol), and DMF (10 mL) was heated at 70 °C for 48 h under argon. The resulting heterogeneous mixture was filtered and the filtrate concentrated under reduced pressure. The concentrated solution was precipitated into water. The cream-colored solid product (1.39 g, 83%) was collected by filtration, washed with water and CH_2Cl_2, and dried in vacuo.

Poly(styrene−*p*-(methyldiaminotriazine)styrene−*p*-(methoxymethyl)styrene, co-PS−$CH_2C_3N_5H_4$/ CH_2OCH_3 (1). A solution of polymer (**3**) (0.76 g, 0.14 mmol), dicyandiamide (0.525 g, 6.2 mmol), and KOH (0.078 g, 1.4 mmol) in 1-propanol (6 mL) was refluxed for 20 h. A precipitate formed as the reaction proceeded. After evaporating 1-propanol under reduced pressure, the resulting crude product was stirred in boiling water for 30 min. The solution was cooled to room temperature and then filtered to collect a cream-colored solid (0.768 g, 87%). Anal. Calcd: C, 78.18; H, 7.08; N, 12.00. Found: C, 77.38; H, 7.00; N, 10.46.

GPC. All comparison, molecular weight values were obtained through use of calibration curves. Gel permeation chromatography was performed on a PLgel mixed-E column (3 μm, 7.5 × 300 mm, Polymer Laboratories Ltd.), using polystyrene as standard, THF and $CHCl_3$ as solvents, and a flow rate of 1 mL/min, with detection at 254 nM.

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Supporting Information Available: ^{1}H NMR, IR, and GPC for polymer **1** and precursors; ^{1}H NMR and IR for monomer **4**; ^{1}H NMR titration curves and data for the polymer **1**−flavin **5** and the monomer **4**−flavin **5** complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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