Exquisite regulation of supramolecular equilibrium polymers in water: Chain stoppers control length, polydispersity and viscoelasticity

Journal:	*Polymer Chemistry*
Manuscript ID	PY-ART-08-2018-001146.R1
Article Type:	Paper
Date Submitted by the Author:	27-Sep-2018

Complete List of Authors:	Karunakaran, Suneesh; Chemistry Department, Georgia Institute of Technology Cafferty, Brian; Chemistry Department, Georgia Institute of Technology Pelaez-Fernandez, Miguel; Georgia Institute of Technology, School of Physics Neselu, Kasahun; Georgia Institute of Technology, School of Biological Sciences Schmidt-Krey, Ingeborg; Georgia Institute of Technology, School of Biological Sciences Fernandez-Nieves, Alberto; Georgia Institute of Technology, School of Physics Schuster, Gary; Georgia Institute of Technology, School of Chemistry and Biochemistry Hud, Nicholas; Chemistry Department, Georgia Institute of Technology

Polymer Chemistry

RSCPublishing

Paper

Exquisite regulation of supramolecular equilibrium polymers in water: Chain stoppers control length, polydispersity and viscoelasticity†

Cite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Suneesh C. Karunakaran,[a] Brian J. Cafferty,[a,d] Miguel Peláez-Fernández,[b] Kasahun Neselu,[c] Ingeborg Schmidt-Krey,[c] Alberto Fernandez-Nieves,[b] Gary B. Schuster,[a] and Nicholas V. Hud*[a]

Supramolecular polymers are increasingly important materials for applications that require the controlled assembly and disassembly of structures having well-defined shapes and properties. Subtle changes in molecular structure and solvent properties can profoundly affect these materials because of the relatively weak non-covalent interactions that govern their formation. We report the discovery that positively charged small molecules that are planar, or have planar elements, can be employed as noncovalent chain stoppers (NCSs) to control the length of supramolecular polymers in aqueous solution. The supramolecular polymers are composed of 2,4,6-triaminopyrimidine and a modified cyanuric acid, monomers that assemble through interactions that mimic the base pairs of DNA. These assemblies are *equilibrium polymers*, a type of supramolecular polymers that, in the absence of any chain stoppers, are extremely long (e.g., multiple microns in length). The lengths of these assemblies are controlled by a NCS in a concentration-dependent and compound-specific manner, giving rise to supramolecular polymers with low polydispersity. This behavior is attributed to the homogeneity of the NCS distribution within the system, and their "ideal-gas-like" number density fluctuations. These supramolecular polymers form hydrogels with viscoelastic properties that can be tuned by the addition of a NCS, thereby demonstrating the potential of this approach for controlling the dimensions of supramolecular polymers in water as well as the macroscopic properties of materials formed by such polymers.

Introduction

The dynamic nature of noncovalent supramolecular polymers imparts on these systems properties that set them apart from covalent polymers.[1] Progress has been made in formulating noncovalent polymers with various architectures, such as spheres, rods and cylinders with increasing complexity through the rational design of molecular building blocks.[2-4] The characteristic dimensions of these polymers (e.g., width, length, symmetry) determine, in part, their properties and functionalities at molecular and macroscopic scales. The ability to control these properties is important for the development of materials for biomedical applications, which often requires polymers that form well-defined structures in water.[5, 6] However, the ability to control the length and polydispersity of supramolecular polymers has not reached the level achieved for covalent polymers, particularly in aqueous media.[7] Controlling the length of supramolecular polymers could enable the creation of soft materials with more-defined macroscopic properties and thereby expand the utility of these materials.[7-10]

Strategies to control supramolecular polymer lengths include the addition of monomers that frustrate growth,[11, 12] kinetic control of polymerization,[13, 14] varying the enantiomeric excess of chiral monomers,[15] homogeneous seeding,[16] and tailored initiators.[17] Most of these strategies involve altering monomer composition or regulating initiation or polymerization. The most well studied strategy for controlling supramolecular polymer length is the use of molecules that compete with monomers to inhibit polymer extension; termed "chain stoppers".[18-22] In this approach, the polymerization of bifunctional monomers is typically controlled by adding a molecule that resembles the assembling monomers but lacks a hydrogen bonding motif[22-25] or metal-coordination center[26, 27] necessary for bidirectional extension of the supramolecular polymers.

Supramolecular polymers that assemble in water from small molecules having planar, hydrophobic surfaces include bicylic Janus heterocycles,[28-30] 2,4,6,-triaminopyrimidine and cyanuric acid, melamine and barbituric acid,[31, 32] all of which assemble as stacked hexads, as well as guanosine and its derivatives, which assemble as stacked tetrads with the aid of cation coordination.[33] These assemblies create large planar surfaces of six or four monomers whose exposure to water is energetically very unfavorable. Thus, the sequestration of these surfaces away from water by stacking drives the formation of long supramolecular polymers.[7, 32, 34, 35] These polymers can be classified as *equilibrium polymers* that are under exclusive thermodynamic control. As such, it is expected that changes in conditions, e.g., temperature and concentration, govern the number of polymer chains and assembled monomers. Significantly, theoretical studies of equilibrium polymers reveal that they may form a single chain of effectively infinite length.[36-39] When monomer and short chain cyclization is not possible or highly disfavored, equilibrium polymer length depends simply on the free energies of monomer-monomer association and polymer-end exposure to solvent.[37, 38] We recently reported that hexads composed of three molecules of 2,4,6-triaminopyrimidine (**TAP**) and three molecules of a hexanoic acid substituted cyanuric acid (**CyCo6**) assemble into multi-micron length supramolecular polymers (Fig. 1).[40] In addition to a general interest as supramolecular structures, these and related assemblies are also of special interest as possible precursors of nucleic acid polymers in the origin of life.[41-46] Here, we report the discovery that **TAP**-**CyCo6** polymers are equilibrium polymers whose lengths can be precisely controlled with chain stoppers.

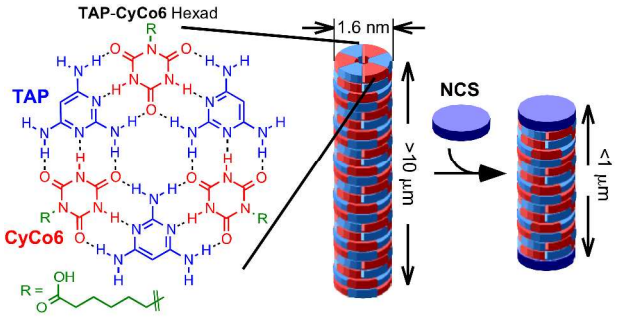


Fig. 1. Chemical structures of **TAP** and **CyCo6** (R = hexanoic acid chain), within the hexad structure formed by H-bonding of three monomers of each. The linear supramolecular polymers formed by the stacking of **TAP**-**CyCo6** hexads are shown schematically, as well as our proposed model that small molecules shorten these polymers by acting as noncovalent chain stoppers (NCSs). The width of 1.6 nm, shown on columnar stack, is estimated for the heterocycle core of the hexad and does not include width added by the hexanoic acid tails of **CyCo6**.

To our knowledge, chain stoppers have been exclusively designed for systems that assemble in organic solvents.[47] Accordingly, previously reported strategies for the design of chain stoppers are not optimal for water-based systems because the less directional hydrophobic effect is often the dominant force for self-assembly in water. That the equilibrium polymerization of **TAP**-**CyCo6** assemblies is driven by the sequestration of hydrophobic surfaces suggests that *any* molecule that decreases the hydrophobicity of the polymer's ends could act as a chain stopper in water. Here we report a method for controlling the length of **TAP**-**CyCo6** equilibrium polymers that uses small molecules as noncovalent chain stoppers, an approach that may be general for water-soluble supramolecular polymers that are formed through π-stacking/hydrophobic interactions and thereby controlling the macroscopic properties of materials formed by such supramolecular polymers.

Experimental

Sample Preparation. For all reported experiments, supramolecular assemblies were prepared by mixing molar equivalents of **CyCo6** and **TAP** in 200 mM sodium phosphate buffer, pH 7. Unless otherwise noted, the preparation of assemblies and all experiments were carried out at 20 °C. **CyCo6** was synthesized using a previously reported procedure (see below) and its purity was determined through NMR spectroscopy and by LCMS. pH measurements were made with a VWR 8100 pH meter equipped with an InLab semi-micro combination electrode.

NMR, UV, and fluorescence spectroscopy. NMR analysis was performed in D_2O on a Bruker DRX-500 NMR and the resulting spectra are the sum of 32 or 64 transients. Samples of **TAP**-**CyCo6** assemblies for investigation by NMR were prepared in 90% H_2O/10% D_2O, and the spectra were acquired using the WATERGATE pulse sequence. 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) was used as an internal standard, which does not show any indication of interacting or being incorporated within the assemblies.

UV-vis spectra were collected using an Agilent 8453 spectrophotometer equipped with an 89090A temperature controller. Cells of different path lengths (0.1 and 0.01 mm) were used depending on the concentration of the sample to maintain an optical density below 1.2. Fluorescence analyses were carried out using a Horiba Jobin Yvon Fluorolog 3-2iHR1 fluorometer.

AFM Imaging and Polymer Length Determination. AFM images were obtained with a Nanoscope IIIa (Digital Instruments) in tapping mode using Si tips (Vistaprobes, 48 N/m) on freshly cleaved mica that was pre-activated by incubation with $MgCl_2$ for 1-2 h. The mica substrate was rinsed with water and dried under N_2. A 2 μl sample of the assembly solution was spread over the mica using N_2 flow and was dried with N_2 gas. The average lengths of chain stoppered supramolecular polymers were calculated by measuring the contour lengths of 60 to 100 supramolecular fibers in the AFM images using the image analysis software Image J (NIH).

Rheological measurements. Rheological measurements were carried out using a Physica MCR 501 rheometer (Anton Paar). The storage modulus, G', and loss modulus, G'', were measured in oscillatory tests at a constant angular frequency of 1 rad/s while sweeping the strain. Frequency scans were performed under a strain of 1.0%. The viscosity measurements were obtained by fitting the slope on the stress-shear rate curves generated by controlled shear rate experiments, for low enough strains. Constant shear stress and shear rate were both imposed to ensure consistency. All measurements were temperature controlled with a Peltier plate at 20 °C.

Dynamic light scattering. Dynamic light scattering experiments were carried out using DynaPro MS/X dynamic light-scattering instrument (Proterion, Piscataway, NJ) with a laser of wavelength 830 nm and a constant scattering collection angle of 90° at room temperature. Each measurement reported is the average of twenty 20 s scattering intensity accumulations.

Materials. 2,4,6-triaminopyrimidine (**TAP**) was purchased from Acros Organic and was used as received. Synthesis of 1-(5-Carboxypentyl)-1,3,5-triazin-2,4,6-trion (**CyCo6**) was performed by slight modification of the procedure reported by Hager et al.[48] **CyCo6**: ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.27 (m, 2H; CH_2), 1.50 (m, 4H; CH_2), 2.19 (t, J = 7.5 Hz, 2H; CH_2CO), 3.61 (t, J = 7.5 Hz, 2H; CH_2N), 11.63 ppm (brm, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 24.1, 25.6, 27.05, 33.5, 40.2, 148.6, 149.8, 174.4 ppm; HRMS m/z calculated for [M - H]-, 242.0777; found, 242.0785. (First reported in Cafferty *et al.*, *Chem. Sci.*, 2014, **5**, 4681-4686)

Results and discussion

DNA binders as chain stoppers of supramolecular polymers.

Our observations that **TAP**-**CyCo6** polymers do not self-cyclize, are several microns in length, and form shear-thinning, reversible hydrogels strongly indicate that these are equilibrium polymers.[40,32] Long-standing theories describing equilibrium polymers[37-39] identify a minimum assembly concentration (MAC) that is analogous to the critical micelle concentration (CMC) of surfactants.[37, 38] Below the MAC, **TAP** and **CyCo6** exist exclusively as free monomers. Above the MAC, monomers coexist in equilibrium with supramolecular polymers that have the potential to become extremely long, approaching a single continuous polymer of infinite length.

As initial candidates for noncovalent chain stoppers (NCSs) of **TAP**-**CyCo6** polymers, we explored positively charged, planar, polycyclic DNA binding agents. The similarity of **TAP** and **CyCo6** to DNA bases suggested that these compounds would interact with the hexads at the ends of these supramolecular polymers forming electrostatically charged termini having more favorable interactions with water than bare, uncapped hexad surfaces.

We initially focused on two compounds, ethidium bromide (**EB**) a monocationic dye that binds duplex DNA, and 5,10,15,20-tetra(*N*-methyl-4-pyridyl)porphine (**TMPyP**) a tetracation that binds G-quadruplexes (Fig. 2A).[33] The addition of just 0.015 mM **TMPyP** to a gelled solution of **TAP**-**CyCo6** (30 mM in each monomer) results in the loss of elastic strength and a decrease in viscosity, effectively fluidizing the system (Fig. 2B). **EB** gives similar results at higher concentration. Specifically, the elastic strength of the hydrogel is reduced by the addition of 0.25 mM **EB** and at 1 mM **EB** there is complete loss of gelation (Fig. 2B).

In addition to **EB** and **TMPyP**, we investigated planar compounds that vary in size, shape and charge for their ability to disrupt water gelation by **TAP**-**CyCo6** polymers (Fig. 2A). Our results indicate that planar, cationic compounds are useful for this purpose, whereas anionic molecules do not destabilize the hydrogel matrix (Fig. 2C). Destabilization of the hydrogel matrix could be due to i) supramolecular polymer shortening, whereby the molecules would be acting as chain stoppers, ii) reduction in the degree of **TAP**-**CyCo6** assembly, and/or iii) disruption of noncovalent polymer cross-linking.[49]

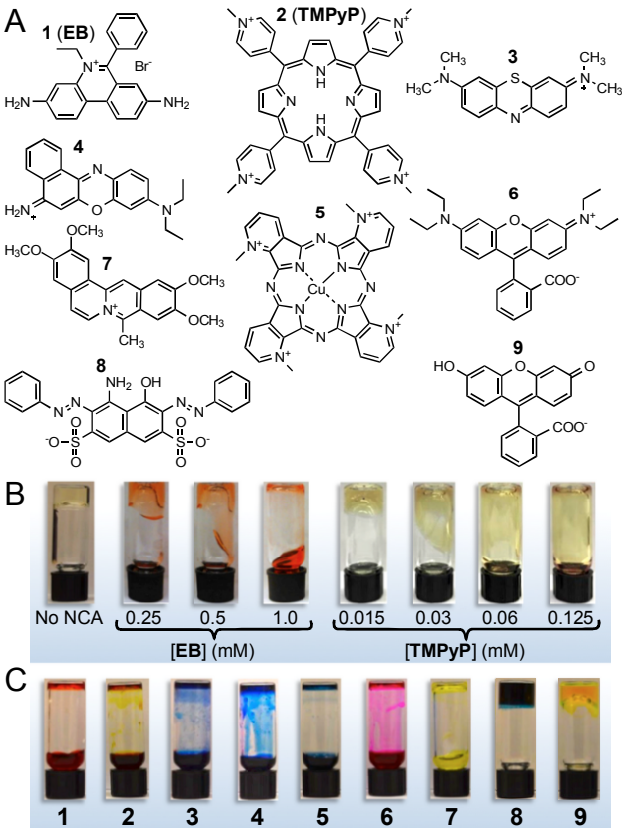


Fig. 2. (A) Small molecules examined in this study as potential noncovalent chain stoppers (NCSs): **1**) ethidium bromide (**EB**); **2**) 5,10,15,20-tetra(*N*-methyl-4-pyridyl)porphine (**TMPyP**); **3**) methylene blue; **4**) Nile blue; **5**) cuprolinic blue; **6**) rhodamine (shown in its zwitterionic form) B; **7**) coralyne; **8**) naphthol blue black; and **9**) fluorescein. (B) Inverted vial test illustrating the concentration-dependent disruption of the **TAP**-**CyCo6** gel by **EB** and **TMPyP**. **TAP** and **CyCo6** were 30 mM in all samples. (C) Inverted vial test containing **TAP**-**CyCo6** supramolecular polymers with the addition of the small molecules shown in A. All samples contained 30 mM of **TAP** and **CyCo6** and 2.5 mM of the small molecule as indicated by compound number.

AFM images reveal a reduction in the length of **TAP**-**CyCo6** supramolecular polymers proportional to **TMPyP** concentration (Figs. 3A-E), supporting the chain stopper mechanism. Measurements of polymers by AFM provide average lengths of 1260, 460, 180, and 110 nm (Fig. 3F) with narrow polydispersity (Figs. S1-S8†) when concentrations of 0.008, 0.015, 0.031 and 0.062 mM **TMPyP**, respectively, are added to **TAP**-**CyCo6** hydrogels. This reduction in length was observed when **TMPyP** was added before or after formation of the **TAP**-**CyCo6** polymers (Fig. 4A,B), consistent with equilibrium control of the polymers and their interaction with the NCS. Transmission electron microscopy (TEM) provided results similar to those of AFM for **TAP**-**CyCo6** polymers in the absence and presence of **TMPyP** (Fig. 4C-F), confirming that the observed change in supramolecular polymer length is not an artefact of AFM sample preparation. Similar experiments with **EB** also revealed its ability to control polymer length. For example, when 1 mM of **EB** is added to a gelled solution of 30 mM **TAP** and **CyCo6**, the supramolecular polymers are observed to have an average length of 480 nm with low dispersity (Fig. S9†). Higher **EB** concentrations result in shorter polymers. For example, increasing the **EB** concentration 2.5 mM reduced the average polymer length to 270 nm (Fig. S10†). These experiments demonstrate that the addition of these DNA binders to **TAP**-**CyCo6** samples alters the length of the supramolecular polymers in a concentration-dependent manner.

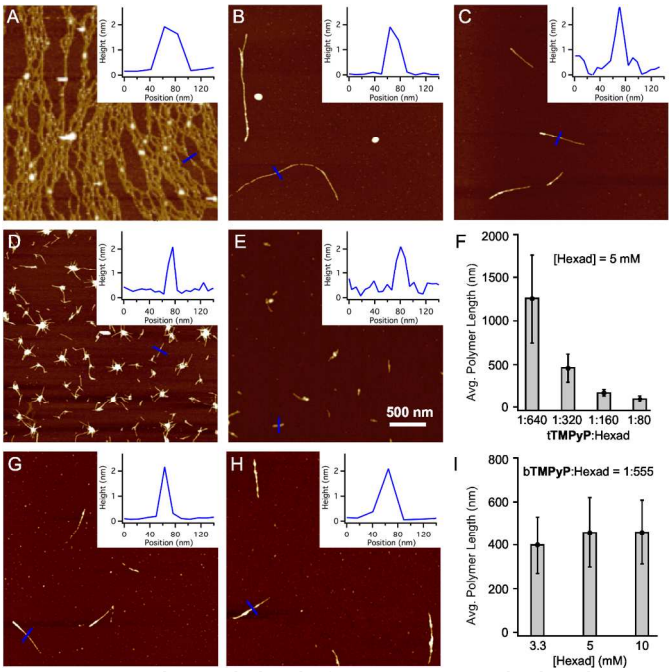


Fig. 3. AFM images showing the length of **TAP**-**CyCo6** supramolecular polymers in the absence and the presence of **TMPyP**. (A) **TAP**-**CyCo6** supramolecular polymers, from a sample 30 mM in each monomer, in the absence of **TMPyP**. Based on a minimal assembly concentration of 15 mM, this sample has 5 mM assembled **TAP**-**CyCo6** hexads. (B-E) Same preparation of **TAP**-**CyCo6** supramolecular polymers as in A, but in the presence of 0.008, 0.015, 0.031 and 0.062 mM t**TMPyP**, respectively. t**TMPyP** = total **TMPyP** in sample, free and bound to hexads. Inserts show heights measured along the blue lines indicated in main panels. (F) Dependence of **TAP**-**CyCo6** polymer lengths on t**TMPyP**:hexad ratio. (G, H) Samples were prepared to have the same ratio of *bound* **TMPyP**:hexad (b**TMPyP**:Hexad) as the sample shown in panel C, which was determined by fluorescence measurements to have 9 μM of bound **TMPyP**. The polymers shown in panel G were prepared by adding 12 μM **TMPyP** to a sample containing 25 mM in **TAP** and **CyCo6** (3.3 mM assembled hexad, and predicted to have 6 μM of bound **TMPyP**). The polymers shown in panel H were prepared by adding 24 μM **TMPyP** to a sample containing 45 mM in **TAP** and **CyCo6** (10 mM assembled hexad, and predicted to have 18 μM of bound **TMPyP**). I) **TAP**-**CyCo6** polymer lengths measured for three samples with the same bound **TMPyP**:hexad ratio, but for three different concentrations of assembled **TAP**-**CyCo6** hexads (samples panels C, G, and H).

Destabilization of the hydrogel matrix by an NCS might also be due to a reduction in the extent of polymerization, which would manifest as an increase in the MAC for **TAP** and **CyCo6**.[49] Solution state ^{1}H NMR spectroscopy can be used to determine the MAC of supramolecular polymers because only the resonances of unassembled monomers are observed.[32, 50] Monomers incorporated into polymers are "invisible" because they tumble so slowly that their resonances are broadened to baseline. ^{1}H NMR measurements indicate that the MAC of **TAP** with **CyCo6** (20°C, 15 mM for each monomer[40]) is unchanged by the addition of **TMPyP** or **EB** (Fig. 5). The ^{1}H NMR analysis reveals that 15 mM **CyCo6** is free in solution. Thus, 15 mM of each monomer is incorporated into supramolecular polymers confirming that although **TMPyP** and **EB** shorten the polymers they do not alter the total number of assembled **TAP** and **CyCo6** monomers (N_t). In the presence of **TMPyP** or **EB**, the same amount of **TAP** and **CyCo6** monomers are assembled into a given number of polymers, N_p, of average length $\langle L \rangle \sim b\langle N_m \rangle$, with b being monomer thickness (along the polymer axis; ~0.34 nm for stacked π systems) and $\langle N_m \rangle$ the average number of monomers per polymer. Since $N_t = \langle N_m \rangle N_p$ is constant, as the polymers shorten, their number must increase proportionally.

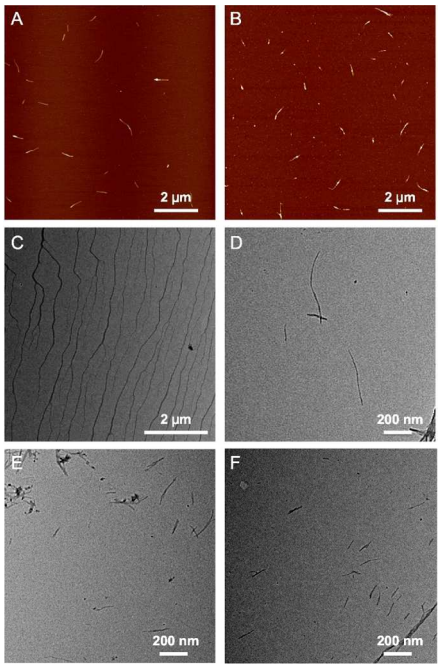


Fig. 4. (A-B) AFM images of **TAP-CyCo6** polymers in the presence of 15 μM **TMPyP** (A) added before gelation and (B) after gelation. (C-F) TEM images of **TAP-CyCo6** supramolecular polymers in the presence of (C) 0; (D) 15; (E) 31 and (F) 62 μM **TMPyP**. All solutions contained 30 mM of **TAP** and **CyCo6**, which, under the conditions of sample preparation, corresponds to 15 mM assembled **TAP** and **CyCo6** (i.e., 5 mM in hexad).

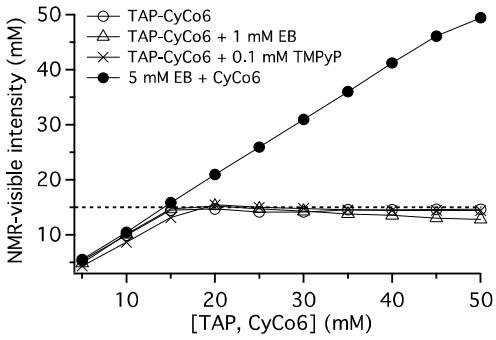


Fig. 5. Determination of the minimal assembly concentration (MAC) for **TAP** and **CyCo6** in the absence and presence of **EB** and **TMPyP**. Apparent solution-phase concentration of **CyCo6** as determined by integration of the methylene ^{1}H NMR signals versus actual **CyCo6** concentration in 1:1 solution with **TAP** in the absence a cationic ligand, in the presence of 1 mM **EB**, and in the presence of 0.1 mM **TMPyP**. A sample with **CyCo6** and 5 mM **EB** without **TAP** is also shown to illustrate the linear response obtained when polymers are not present and that **EB** does not alter the integration of free **CyCo6** methylene resonances. The plateau of signal intensity for samples containing **TAP** and **CyCo6** indicate a MAC of 15 mM. For B, all measurements were carried out at 20 °C. Sample ^{1}H NMR spectra from which the MAC determination plots were made are provided in Fig. S15†.

TMPyP association constant (K_a) and its consistency with the Chain Stopper mechanism.

Theoretical models have been developed to predict the impact of chain stoppers on the equilibrium length of supramolecular polymers formed in organic solvents.[18, 23, 25, 51-54] According to the formalism used by Knoben *et al.*,[23] the average degree of polymerization in the presence of a chain stopper is

$$N_x = \frac{N_o n}{n(1-x)+N_o x} \qquad \text{(Eq. 1)}$$

where N_o is the degree of polymerization in the absence of chain stoppers (average number of monomers per polymer chain), n is the number of chain stoppers associated with each polymer chain, and x is the ratio of chain stoppers in the sample to the sum of assembled monomers and chain stoppers. Knoben *et al.* used this equation to predict the impact of chain stoppers on the length of supramolecular polymer chains in a system for which the initial degree of polymerization could be determined and for which it could be assumed that all added chain stoppers were bound to the ends of the chains. In the present study, the initial length of **TAP-CyCo6** supramolecular polymers is several microns, but a precise measurement is not possible because the assemblies are too long for quantitative solution phase measurements (e.g., by NMR or dynamic light scattering) and because interpolymer associations obscure the ends of the assemblies in AFM images (Fig. 3A). Additionally, unlike the system modelled by Knoben *et al.*, we cannot assume that all added chain stoppers are associated with the supramolecular polymers (see below).

By rearranging the Eq. 1, the concentration of noncovalent chain stoppers bound to the polymers, NCS_b, can be expressed in terms of the initial degree of polymerization, N_o, and the degree of polymerization in the presence of chain stoppers, N_x (Eq. 2).

$$NCS_b = \frac{C_{as}}{\frac{N_o-1}{\frac{n}{N_o-1}}-1} \qquad \text{(Eq. 2)}$$

In this equation C_{as} is the concentration of assembled hexads of **TAP** and **CyCo6**, n is again the number of chain stopper molecules associated with each polymer chain or, equivalently, the number of chain stopper molecules needed to split an existing polymer chain into two separate supramolecular polymers. For samples containing 30 mM of **TAP** and **CyCo6** at 20 °C, C_{as} = 5 mM (i.e., 15 mM in assembled **TAP** and **CyCo6** monomers). When 31 μM **TMPyP** is added to this sample, an average polymer length of 180 nm is measured by AFM (Fig. 3F). From these experimental parameters for C_{as} and N_x, and by assigning a binding ratio of one **TMPyP** molecules per polymer end (i.e., n = 2), Eq. 2 provides estimates of the concentration of bound **TMPyP** from a plot of NCS_b as a function of N_o (Fig. 6). Explicitly, converting N_o to polymer length (based on an axial thickness of 0.34 nm per hexad) results in calculated values of NCS_b that range from a minimum for an initial polymer length of 5 microns to a maximum for supramolecular polymers of infinite length. As shown in Fig. 6, even for this range of possible initial polymer lengths that is only defined by a lower bound, the calculated possible concentration of bound **TMPyP** is restricted to the narrow range between 18.3 μM and 19 μM.

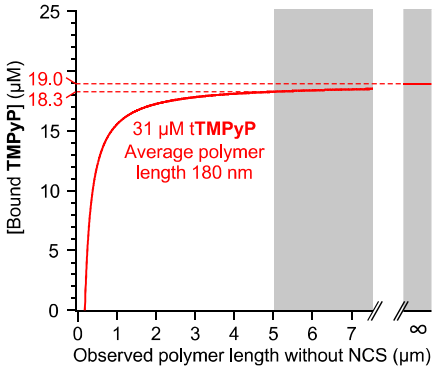
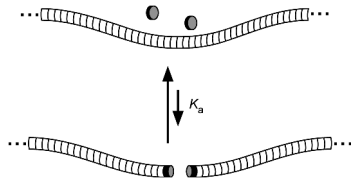


Fig. 6. Plot of calculated concentrations of bound **TMPyP** (vertical axis) as a function of initial polymer length (in the absence of chain stoppers) as predicted by Equation 2 for the average polymer length of 180 nm observed for a sample containing a *total* **TMPyP** concentration (t**TMPyP**) of 31 μM. The **TAP** and **CyCo6** concentration was 30 mM, which corresponds to an assembled hexad concentration of 5 mM (i.e., 15 mM in assembled **TAP** and **CyCo6**). For these

conditions and parameters, in the absence of any NCS, the observed length of **TAP-CyCo6** polymers is greater than 5 microns (region indicated by gray shading). If one **TMPyP** molecule is assigned to each polymer end (i.e., two per polymer), the curve reveals the concentration of *bound* **TMPyP** must be between 18.3 and 19 μM.

The concentration of bound **TMPyP** for the conditions described in Fig. 6 can be used to calculate its apparent association constant (K_a) with **TAP-CyCo6** hexads. For this approach, we consider that **TMPyP** exists in equilibrium between two states; free in solution or bound to a hexad surface at the end of a stack (Scheme 1). Further, the hexads are in equilibrium between two states; either having both "faces" stacked with other hexads within an essentially infinitely long assembly or, for a hexad at the end of a linear assemblies, having one face stacked against a **TMPyP** (Scheme 1). The equilibrium equation for this system can be written simply as Eq. 3.



Scheme 1: Proposed equilibrium model for NCS interaction with stacked **TAP-CyCo6** hexads. The ••• on each side of the hexad stacks indicates that these supramolecular polymers, which are *equilibrium polymers*, are extremely long (essentially infinite) in the absence of bound NCS.

[free **TMPyP**] + [**TAP-CyCo6** hexad surfaces] ⇌ [**TMPyP** bound to hexads]
(Eq. 3)

We calculated K_a using Eq. 3 from the concentration of bound **TMPyP** determined above (19 μM), the concentration of free **TMPyP** (12 μM: total **TMPyP** in the sample minus bound **TMPyP**), and the total concentration of hexad surfaces (or potential **TMPyP** binding sites) (10 mM: 2 x hexad concentration). Based on these values, K_a = 19 μM/(12 μM*10 mM) ≈ 160 M^{-1}. This calculation shows that despite a low association constant, **TMPyP** and other analogous NCS can have a profound effect on the lengths of these non-covalent polymers.

A second, completely independent, experimental method was also used to determine the apparent **TMPyP**–hexad association constant based on the change in **TMPyP** fluorescence upon binding to **TAP-CyCo6** hexads. As shown in Fig. 7, the fluorescence intensity of samples containing 31 μM **TMPyP** decreases as the concentration of assembled **TAP-CyCo6** hexads is increased from 0 to 28.3 mM assembled hexad. A least-squares fit of this fluorescence intensity data with a simple equilibrium binding model of Eq. 3 reveals a K_a ≈ 130 M^{-1} for **TMPyP** binding to **TAP-CyCo6** hexads.

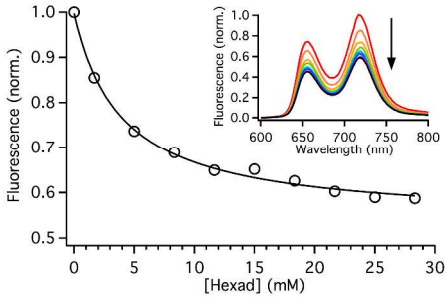


Fig. 7. Fluorescence intensity measurements and determination of K_a for the binding of **TMPyP** to **TAP-CyCo6** hexads. (Inset) Fluorescence emission spectra of 0.031 mM **TMPyP** in the presence of **TAP** and **CyCo6**, at equal concentrations, from 10 mM (red trace) to 100 mM (black trace) in steps of 10 mM. Spectra were normalized to the intensity of 10 mM **TAP** and **CyCo6** sample at 718 nm. (Main panel) Plot of **TMPyP** fluorescence at 718 nm, normalized, as a function of **TAP-CyCo6** hexad concentration. For the conditions of this study, the **TAP-CyCo6** minimal assembly concentration (MAC) was determined to be 15 mM in **TAP** and **CyCo6**. Thus, the first data point from a sample 10 mM in **TAP** and **CyCo6** contains free monomers, but no assembled hexads. The hexad concentration of all other samples is determined by the equation [Hexad] = ([**TAP**, **CyCo6**] – 15 mM)/3. The line is the result of fitting the fluorescence data (open circles) with an equation for equilibrium binding of **TMPyP** to hexads, which revealed a K_a of 130 M^{-1}.

The excellent agreement between these two calculations of K_a for **TMPyP** binding provides confidence in the accuracy of this value as well as in the applicability of the formalism of Knoben *et al.* to this system. As an additional validity test and measurement of the K_a for **TMPyP** binding, we measured the lengths of **TAP-CyCo6** polymers in the presence of two-fold lower and two-fold greater concentrations of **TMPyP**. These samples again contained 15 mM of *assembled* **TAP** and **CyCo6** (5 mM hexad). Based on the K_a value determined above, a sample containing a total **TMPyP** concentration of 15 μM is predicted to have 9 μM bound **TMPyP**, and therefore an average polymer length of 350 nm (i.e., an average of 6200 monomers per chain). For this sample we observe an average polymer length of 460 nm (Fig. 3C). For a sample with the same **TAP** and **CyCo6** concentrations, but 62 μM in total **TMPyP**, the concentration of bound **TMPyP** is predicted to be 38 μM with an average polymer length of 90 nm. For that sample we observe an average polymer length of 110 nm (Fig. 3E). These observed average polymer lengths (within 25% of the predicted values) provide further confirmation of the validity of the equilibrium chain stopping mechanism model by which **TMPyP** shortens **TAP-CyCo6** polymers. The measured polymer lengths determined from AFM images, from which average polymer lengths were determined, are provided in Table S1†.

In addition, the model proposed by Knobel *et al.*, predicts that at relatively high monomer concentrations the degree of polymerization and the average chain length of the supramolecular polymers will be independent of the monomer concentration and only depend on the chain stopper fraction. We therefore also tested the applicability of this model to our system by measuring the length of the **TAP-CyCo6** supramolecular polymers at various hexad concentrations all

at that same ratio of assembled hexad to bound NCS. For the sample discussed above containing 5 mM of **TAP**-**CyCo6** hexads and 15 μM of total added **TMPyP** (bound **TMPyP** is calculated to be 9 μM), we observe an average polymer length of 460 nm. Significantly, we observe polymers having essentially the same average lengths (400 nm and 460 nm, respectively) for samples predicted to contain 3.3 mM and 10 mM of assembled **TAP**-**CyCo6** hexads with 6 μM and 18 μM of bound **TMPyP**, respectively (Figs. 3G-I). Clearly, this system follows the model of Knobel *et al*. Similar results are obtained for **EB**, but they are more complex to interpret because up to three **EB** molecules may be bound at each chain end. Nevertheless, for **EB** we estimate $K_a \approx 1.5\ M^{-1}$ (See ESI†), a value much smaller than for **TMPyP**, which is consistent with the idea that cationic charge and hydrophilic surface area are primarily contributors to binding.

The association constants of **TMPyP** and **EB** with **TAP**-**CyCo6** hexads are surprisingly small compared with the association constant of these molecules with DNA. Specifically, the association constant reported for **EB** binding to duplex DNA is almost 10^5 times greater than that measured here for binding to **TAP**-**CyCo6** hexads.[55] Also, the association constant of **TMPyP** for G-quadruplex DNA and to duplex DNA is about 40-fold greater than measured here for **TMPyP** binding to **TAP**-**CyCo6** hexads.[56, 57] Thus, even though only a small fraction of hexads in the supramolecular polymers are associated with these chain stoppers, they exhibit a powerful effect on polymer length. The exposure of one face of these planar, charged molecules to water must be far more favorable energetically than exposing one surface of a **TAP**-**CyCo6** hexad to water (consider the good water solubility of **TMPyP** and **EB** versus the extreme hydrophobicity of **TAP**-**CyCo6** hexads[40]). Thus, the binding of these small molecules to hexads within the supramolecular polymers create positions along the polymers where the 'breaking' of the polymer results in the exposure NCS surfaces water, which is much more favorable energetically than the exposure of bare hexad ends (Scheme 1).

Polydispersity in length control using DNA binders as chain stoppers.

We now consider the physical origins of the relatively low polydispersity observed for **TAP**-**CyCo6** polymers in the presence of NCSs. Our explanation begins with the observation that the MAC of **TAP** with **CyCo6** is not altered by the addition of a NCS. As discussed above, under conditions where the length of **TAP**-**CyCo6** polymers is effectively infinite (*i.e.*, ≥10 μm) the concentration of polymer ends is defined by the concentration of NCSs bound to **TAP**-**CyCo6** hexads, which is also an equilibrium value defined by NCS concentration, hexad concentration, and K_a for a particular NCS.

We have constructed a model for polymer length and polydispersity that can be viewed as a 1D version of the classical statistical mechanics model for determining the spatial distribution of molecules in an ideal gas.[58] That is, the total number of hexads in a solution is analogous to the total number of molecules in the overall volume of an ideal gas. It is not that the hexads are non-interacting, as is the case of an ideal gas, but the state of a hexad does not depend on the concentration of hexads within a given volume. We consider the number of polymers in a sample into which hexads are distributed to be equivalent to the smaller volumes into which an ideal gas can conceptually be divided. In the case of equilibrium polymers, the number of bound NCSs governs the number of polymers into which hexads are divided. By analogy with the ideal gas model, the mean number of hexads per polymer is equal to the total number of hexads divided by the number of polymers, which in the present case is equal to one half the number of bound NCSs (a conclusion that is consistent with the analysis presented by Knoben *et al*.[23] for when the ratio of end cappers to assembled monomers is <<1). We can further extend our analogy to estimate the standard deviation of the number of hexads per polymer. For ideal gases, the root mean square fluctuations in the number of molecules within a volume element equals the square root of the mean number of molecules in that volume. This implies that the hexad polymer length fluctuations can be approximated by the square root of the mean number of hexads per polymer times the thickness of a hexad ($\sim 0.34\ nm * (\langle N_o \rangle)^{1/2}$). An equation for polymer polydispersity based on this model is derived in the SI. This equation predicts that polymer dispersity should be very small; ideally, 1.002 for **TAP**-**CyC6** polymers with a mean length of 500 nm (or 1500 hexads).

The polydispersity of **TAP**-**CyC6** polymers was determined experimentally by measuring the lengths of 60 to 100 polymers by AFM for samples with various concentrations of **TMPyP** (Figs. S1 to S8†) and **EB** (Figs. S9 and S10†). A representative AFM image showing the traces of polymer contour lengths measured is shown in Fig. S16†, and all lengths measured are provided in Table S1†. The lowest polydispersity measured was 1.044, for sample containing 5 mM assembled hexads and 31 μM **TMPyP**. In this sample the average polymer length is ca. 500 nm; the length used in the model calculation presented above. Although 1.044 is higher than the predicted value of 1.002 for an ideal system, this polydispersity is still relatively small for a synthetic system of self-assembled polymers, and supportive of our method for controlling supramolecular polymer lengths. The polydispersity of polymers controlled by **EB** tends to be larger than those observed for **TMPyP**-capped polymers; values up to 1.17 are observed. It is possible that other small molecules may provide polydispersity values that are intermediate to this range of values measured, and perhaps some can provide even lower polydispersities, as the hydrogel-disruption results shown in Fig. 2D indicate that many compounds are likely to have the ability to control the length of fibers formed by **TAP** and **CyCo6**.

Impact of Noncovalent Chain Stoppers on TAP-CyCo6 hydrogel bulk properties.

Oscillatory rheology measurements in the linear regime were made to quantify the viscoelastic properties of the hydrogels as a function of **TMPyP** and **EB** concentration. We assessed changes in the dynamic moduli of solutions containing 30 mM **TAP** and **CyCo6** in the absence of an NCS and in the presence of **TMPyP** and **EB**, respectively (Fig. 8). At a constant strain, the shear modulus (G′) and the loss modulus (G′′) were plotted as functions of the angular frequency in the range 0.01 to 100 rad/s in Fig. 8A. At high frequency, the storage modulus, G′, is higher than the loss modulus, G′′, indicating a dominance of the elastic response of the **TAP-CyCo6** assemblies. At low frequency, corresponding to relatively long time scales, G′′ is larger than G′, indicating the importance of viscous dissipation. In this regime, the sample flows. The crossover frequency, ω_c, determines the structural relaxation time of the material, $\tau = 2\pi\omega_c$, which quantifies the characteristic time for molecular relaxations; we can think of τ as the time required for the polymer assembly to rearrange and change spatial configuration. In the presence of **TMPyP** (Fig. 8B), ω_c shifts to higher values, indicating that the sample becomes predominately liquid-like over an extended frequency range; note that τ becomes about two orders magnitude smaller. Consistent with this observation, G′ is also decreases by about an order of magnitude within the same frequency range, while G′′ remains approximately the same as when no **TMPyP** is present in solution. Fig. 8C further illustrates that ω_c also considerably increases with **EB** concentration. Dependence of **EB** concentration on the crossover frequency, ω_c is shown in Fig. 8D and individual plots of G′ and G′′ as a functions of angular frequency for additional concentrations of **EB** are provided in Fig. S11†. These oscillatory rheology results are in good agreement with the visual observations, which indicate that the flow behaviour of the **TAP-CyCo6** solutions is proportional to the concentration of both additives, with **TMPyP** "melting" the gel state at much lower concentrations.

Steady state rheology was employed to further investigate the mechanism by which putative NCAs modulate the bulk properties of **TAP-CyCo6** supramolecular polymers. Experiments were performed at constant **TAP-CyCo6** concentration (30 mM each monomer) and various concentrations of **TMPyP** at 20 °C. The addition of **TMPyP** results in a decrease of the low shear viscosity of the **TAP-CyCo6** hydrogels (Fig. S12†), providing additional evidence that the (average) stack length of **TAP-CyCo6** in solution is reduced by the presence of **TMPyP**.[22] Overall, these steady state rheological results are also consistent with expectations for equilibrium polymers whose lengths are controlled by an NCS.[38]

Dynamic light scattering (DLS) experiments also support the observation that both **TMPyP** and **EB** modulate the properties of hydrogels formed by **TAP-CyCo6** assemblies by reducing polymer length in a concentration-dependent manner. In these experiments, the electric-field autocorrelation function, $g_E^2(\tau)$ was determined for data from 30 mM **TAP-CyCo6** solutions containing **TMPyP** and **EB** at concentrations ranging from 0.0034 mM to 2 mM. For both ligands, the characteristic decay of the function changes from a two-step decay indicative of the existence of two well-defined dynamic time-scales in the system, to a single fast decay (Figures S13 and S14†) at higher NCA concentration consistent with having significantly shorter polymer chains.

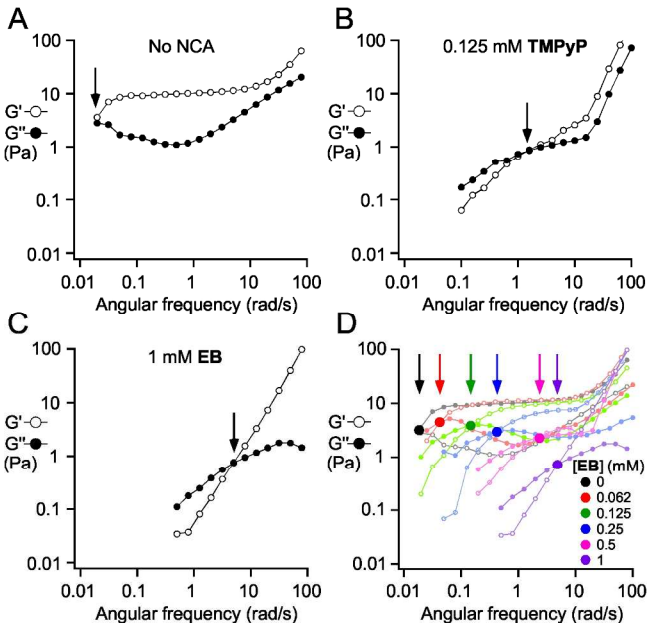


Fig. 8. Rheology analysis of solutions containing **TAP-CyCo6** supramolecular polymers in the presence and absence of **EB** and **TMPyP**. (A-C) Angular frequency dependent dynamic shear storage modulus (G′) and loss modulus (G′′) of the **TAP-CyCo6** gel (A) in the absence of **EB** and **TMPyP**, (B**)** in the presence of 0.125 mM **TMPyP**, and (C) in the presence of 1 mM **EB**. (D) The G′-G′′ crossover points at different concentrations of **EB** (0 to 1 mM) indicated by large dots. Horizontal positions of cross over points are indicated with arrows. All samples contained 30 mM of **CyCo6** and **TAP**.

Conclusions

We have shown that **TMPyP** and **EB**, and likely a variety of small molecules that are planar and positively charged, can act as noncovalent chain stoppers (NCSs) for **TAP-CyCo6** polymers in water, even though these molecules have relatively low association constants for the supramolecular polymers. Nevertheless, these molecules can have a dramatic effect on the length of equilibrium polymers. Moreover, under conditions where extremely long equilibrium polymers are favored; a weakly binding NCS can provide polymers of predictable length and low polydispersity. The discovery that a wide variety of small molecules can act as capping agents for the same supramolecular polymer system suggests that this approach is potentially generalizable for controlling the length of water-soluble equilibrium polymers, and their associated

hydrogels, if the polymers are formed by monomers that associate exclusively, or primarily, through the stacking of planar, hydrophobic surfaces.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the NSF and the NASA Astrobiology Program, under the NSF Center for Chemical Evolution (CHE-1504217) (SK, BJC, GBS, and NVH) and project DMR-1609841 (AF-N), and the NASA Exobiology Program NNX13AI02G (SK, MPF, and NVH). We thank L. D. Williams for discussions.

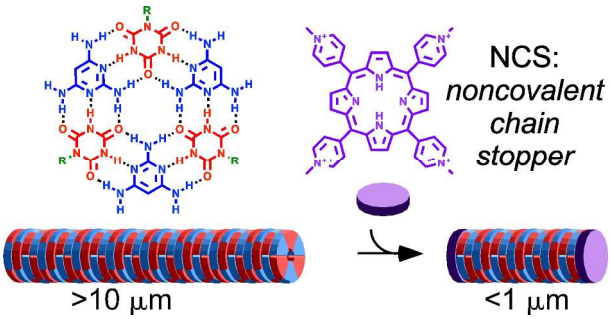
References

[a]Department of Chemistry and Biochemistry, [b]School of Physics, [c]School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia 30332 (USA). [d]Present Addresses: Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA.
*Email: hud@gatech.edu.

† Electronic Supplementary Information (ESI) available: Materials, methods and supplemental images. See DOI: 10.1039/b000000x/

1. T. Aida, E. W. Meijer and S. I. Stupp, *Science*, 2012, **335**, 813-817.
2. J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 2013, **52**, 2836-2850.
3. C. Hunter, *Nature*, 2011, **469**, 39-41.
4. G. M. Whitesides and B. Grzybowski, *Science*, 2002, **295**, 2418-2421.
5. E. Krieg, M. M. C. Bastings, P. Besenius and B. Rybtchinski, *Chem. Rev.*, 2016, **116**, 2414-2477.
6. J. D. Hartgerink, E. Beniash and S. I. Stupp, *Science*, 2001, **294**, 1684-1688.
7. T. F. De Greef, M. M. Smulders, M. Wolffs, A. P. Schenning, R. P. Sijbesma and E. W. Meijer, *Chem. Rev.*, 2009, **109**, 5687-5754.
8. D. van der Zwaag, T. F. A. de Greef and E. W. Meijer, *Angew. Chem. Int. Ed.*, 2015, DOI: 10.1002/anie.201503104, 8334-8336.
9. R. Deng and X. Liu, *Nat. Chem.*, 2015, **7**, 472-473.
10. L. C. Palmer, Y. S. Velichko, M. O. de la Cruz and S. I. Stupp, *Philos. Trans. R. Soc. A-Math. Phys. Eng. Sci.*, 2007, **365**, 1417-1433.
11. P. Besenius, G. Portale, P. H. H. Bomans, H. M. Janssen, A. R. A. Palmans and E. W. Meijer, *Proc. Natl. Acad. Sci. USA*, 2010, **107**, 17888-17893.
12. F. C. Mackintosh, S. A. Safran and P. A. Pincus, *J. Phys.-Condes. Matter*, 1990, **2**, SA359-SA364.
13. S. Ogi, K. Sugiyasu, S. Manna, S. Samitsu and M. Takeuchi, *Nat. Chem.*, 2014, **6**, 188-195.
14. C. Schaefer, I. K. Voets, A. R. A. Palmans, E. W. Meijer, P. van der Schoot and P. Besenius, *ACS Macro Letters*, 2012, **1**, 830-833.
15. J. Kumar, H. Tsumatori, J. Yuasa, T. Kawai and T. Nakashima, *Angew. Chem. Int. Ed. Engl.*, 2015, **54**, 5943-5947.
16. A. Pal, M. Malakoutikhah, G. Leonetti, M. Tezcan, M. Colomb-Delsuc, V. D. Nguyen, J. van der Gucht and S. Otto, *Angew. Chem. Int. Ed. Engl.*, 2015, DOI: 10.1002/anie.201501965.
17. J. Kang, D. Miyajima, T. Mori, Y. Inoue, Y. Itoh and T. Aida, *Science*, 2015, **347**, 646-651.
18. R. A. Sanguramath, P. F. Nealey and R. Shenhar, *Chem.-Eur. J.*, 2016, **22**, 10203-10210.
19. T. Pinault, C. Cannizzo, B. Andrioletti, G. Ducouret, F. Lequeux and L. Bouteiller, *Langmuir*, 2009, **25**, 8404-8407.
20. F. Lortie, S. B. Boileau, L. Bouteiller, C. Chassenieux and F. Laupretre, *Macromolecules*, 2005, **38**, 5283-5287.
21. R. P. Sijbesma, F. H. Beijer, L. Brunsveld, B. J. B. Folmer, J. Hirschberg, R. F. M. Lange, J. K. L. Lowe and E. W. Meijer, *Science*, 1997, **278**, 1601-1604.
22. M. M. J. Smulders, M. M. L. Nieuwenhuizen, M. Grossman, I. A. W. Filot, C. C. Lee, T. F. A. de Greef, A. Schenning, A. R. A. Palmans and E. W. Meijer, *Macromolecules*, 2011, **44**, 6581-6587.
23. W. Knoben, N. A. M. Besseling and M. A. C. Stuart, *Macromolecules*, 2006, **39**, 2643-2653.
24. S. Yagai, T. Iwashima, T. Karatsu and A. Kitamura, *Chem. Comm.*, 2004, DOI: 10.1039/b401132e, 1114-1115.
25. V. Berl, M. Schmutz, M. J. Krische, R. G. Khoury and J. M. Lehn, *Chem.-Eur. J.*, 2002, **8**, 1227-1244.
26. K. Ogawa and Y. Kobuke, *Angew. Chem., Int. Ed. Engl.*, 2000, **39**, 4070-4073.
27. U. Michelsen and C. A. Hunter, *Angew. Chem., Int. Ed. Engl.*, 2000, **39**, 764-767.
28. H. Fenniri, M. Packiarajan, K. L. Vidale, D. M. Sherman, K. Hallenga, K. V. Wood and J. G. Stowell, *J. Am. Chem. Soc.*, 2001, **123**, 3854-3855.
29. H. Fenniri, B. L. Deng, A. E. Ribbe, K. Hallenga, J. Jacob and P. Thiyagarajan, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 6487-6492.
30. H. Fenniri, B. L. Deng and A. E. Ribbe, *J. Am. Chem. Soc.*, 2002, **124**, 11064-11072.
31. B. J. Cafferty, D. M. Fialho, J. Khanam, R. Krishnamurthy and N. V. Hud, *Nature Commun.*, 2016, **7**.
32. B. J. Cafferty, I. Gallego, M. C. Chen, K. I. Farley, R. Eritja and N. V. Hud, *J. Am. Chem. Soc.*, 2013, **135**, 2447-2450.
33. J. T. Davis, *Angew. Chem. Int. Ed. Engl.*, 2004, **43**, 668-698.
34. D. Chandler, *Nature*, 2005, **437**, 640-647.
35. L. Albertazzi, F. J. Martinez-Veracoechea, C. M. A. Leenders, I. K. Voets, D. Frenkel and E. W. Meijer, *Proc. Natl. Acad. Sci. USA*, 2013, **110**, 12203-12208.
36. M. H. Theil, *Macromolecules*, 1969, **2**, 137-142.
37. P. D. Gujrati, *Phys. Rev. B*, 1989, **40**, 5140-5143.
38. M. E. Cates and S. J. Candau, *J. Phys.-Condes. Matter*, 1990, **2**, 6869-6892.
39. J. P. Wittmer, A. Milchev and M. E. Cates, *J. Chem. Phys.*, 1998, **109**, 834-845.
40. B. J. Cafferty, R. R. Avirah, G. B. Schuster and N. V. Hud, *Chem. Sci.*, 2014, **5**, 4681-4686.
41. N. Avakyan, A. A. Greschner, F. Aldaye, C. J. Serpell, V. Toader, A. Petitjean and H. F. Sleiman, *Nat. Chem.*, 2016, **8**, 368-376.
42. C. Li, B. J. Cafferty, S. C. Karunakaran, G. B. Schuster and N. V. Hud, *Physical Chemistry Chemical Physics*, 2016, **18**, 20091-20096.
43. B. J. Cafferty and N. V. Hud, *Isr. J. Chem.*, 2015, **55**, 891-905.
44. M. C. Chen, B. J. Cafferty, I. Mamajanov, I. Gállego, J. Khanam, R. Krishnamurthy and N. V. Hud, *J. Am. Chem. Soc.*, 2014, **136**, 5640-5646.
45. X. J. Piao, X. Xia and D. Bong, *Biochemistry*, 2013, **52**, 6313-6323.
46. N. V. Hud, B. J. Cafferty, R. Krishnamurthy and L. D. Williams, *Chem. Biol.*, 2013, **20**, 466-474.
47. P. Besenius, *J. Polym. Sci. Pol. Chem.*, 2017, **55**, 34-78.
48. K. Hager, A. Franz and A. Hirsch, *Chem.-Eur. J.*, 2006, **12**, 2663-2679.
49. L. J. Fetters, D. J. Lohse, D. Richter, T. A. Witten and A. Zirkel, *Macromolecules*, 1994, **27**, 4639-4647.
50. A. R. Hirst, I. A. Coates, T. R. Boucheteau, J. F. Miravet, B. Escuder, V. Castelletto, I. W. Hamley and D. K. Smith, *J. Am. Chem. Soc.*, 2008, **130**, 9113-9121.
51. M. M. J. Smulders, M. M. L. Nieuwenhuizen, T. F. A. de Greef, P. van der Schoot, A. Schenning and E. W. Meijer, *Chem.-Eur. J.*, 2010, **16**, 362-367.
52. W. Knoben, N. A. M. Besseling and M. A. C. Stuart, *J. Chem. Phys.*, 2007, **126**, 9.

53. W. Knoben, N. A. M. Besseling, L. Bouteiller and A. C. Stuart, *Phys. Chem. Chem. Phys.*, 2005, **7**, 2390-2398.
54. G. Ercolani, *Chem. Comm.*, 2001, DOI: 10.1039/b101678b, 1416-1417.
55. E. D. Horowitz and N. V. Hud, *J. Am. Chem. Soc.*, 2006, **128**, 15380-15381.
56. I. Haq, J. O. Trent, B. Z. Chowdhry and T. C. Jenkins, *J. Am. Chem. Soc.*, 1999, **121**, 1768-1779.
57. J. Ren and J. B. Chaires, *Biochemistry*, 1999, **38**, 16067-16075.
58. D. Yoshioka, *Statistical physics: an introduction*, Springer, Berlin ; New York, 2007.



Positively charged molecules with planar surfaces control the length of supramolecular polymers formed by 2,4,6-triaminopyrimidine and a modified cyanuric acid.