

Emergent mechanical properties of self-assembled polymeric capsules

Ronald K. Castellano^{†*}, Ross Clark^{†§}, Stephen L. Craig^{†*}, Colin Nuckolls^{†*}, and Julius Rebek, Jr.^{†¶}

[†]The Skaggs Institute for Chemical Biology and Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037; and [§]Kelco Biopolymers, 8225 Aero Drive, San Diego, CA 92123

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Synthetic self-assembled systems combine responsiveness and reversibility with the ability to perform chemical tasks such as molecular recognition and catalysis. An unmet challenge is the construction of polymeric materials that, like nature's tubulin, are simultaneously reversible and capable of useful physical tasks. We report here a class of reversibly formed polymers that show covalent-polymer mechanical integrity in solution and in the solid state. Non-Newtonian, polymeric behavior is observed despite the low molecular weight of the individual subunits and the seemingly weak forces holding the assemblies together. These polymers assemble through self-complementary hydrogen bonding and by physical encapsulation of small molecules; accordingly, the emergent macroscopic structure and function can be controlled by appropriate chemical signals.

Reversible polymers offer many advantages over their traditional covalent counterparts because of their benign processing conditions and ability to organize rapidly into ordered systems with few imperfections (1–3). A range of noncovalent polymeric systems based on hydrogen bonding has appeared in recent years, and ordered films and fibers have been produced that display a rich array of structures (4–8). Stadler and coworkers (5, 9) were among the first to examine the solid-state bulk dynamic properties of polymers in which hydrogen bonds partly define the polymer main chain. Likewise, Meijer and coworkers (6) showed that reversible polymers constructed from strongly associating subunits ($K_a > 10^6 \text{ M}^{-1}$) can exhibit increased viscosities in solution and covalent polymer viscoelasticity in the bulk. We have previously used NMR spectroscopy (10) and light scattering to verify the polymeric nature of assemblies composed of reversibly formed encapsulation complexes. All of these polymers are formed from specific interactions that are well-characterized in solution. Although weak, noncovalent forces are known to contribute to polymeric viscoelasticity in other systems, e.g., micelles (11) and organometallic assemblies (12), little is known about the mechanical properties of linear, hydrogen-bonded, main-chain polymers in solution (13). We now report that synthetic reversible polymeric capsules—the polycaps—evince mechanical properties and viscoelastic behavior that are characteristic of covalent polymers.

Materials and Methods

Proton ¹H NMR spectra were recorded on a Bruker (Billerica, MA) DRX-600 (600 MHz) spectrometer. IR spectra were recorded on a Perkin–Elmer Paragon 1000PC Fourier-transform infrared spectrometer. Electrospray ionization mass spectrometry experiments were performed on an API III Perkin–Elmer SCIEX triple quadrupole mass spectrometer. Rheometry measurements were obtained with both parallel-plate and cone-and-plate geometries on a Rheometric Scientific (Piscataway, NJ) SR-5000 dynamic stress rheometer. Rheology studies were performed in *o*-dichlorobenzene, which is both high boiling (180°C) and an excellent guest for the calixarene capsules. Temperature

control was provided by a Peltier heating stage. All reagents were purchased from Aldrich or Fluka and used without further purification. The PAMAM tetraamine (generation 0) was purchased from Aldrich as a 20 wt % solution in methanol. Before coupling, the methanol was removed *in vacuo* and the reagent was dried under high vacuum overnight.

Synthesis of the linear polycap monomer **1** has been previously reported (10). The tetravalent cross-linker **2** was achieved as follows. To a solution of calixarene monoacid (0.26 g, 0.17 mmol; ref. 10) in dimethylformamide (10 ml) was added PyBOP (0.091 g, 0.17 mmol), NEt₃ (39 μl, 0.28 mmol), and the PAMAM tetraamine (0.018 g, 0.035 mmol) as a solution in dimethylformamide (2 ml). The solution was heated to 40°C for 2.5 h and overnight at room temperature. After aqueous work-up, the crude material was purified by preparative TLC (10:1 CH₂Cl₂/MeOH) and methanol precipitation to give 0.055 g (25%) of the pure material as a white powder. ¹H NMR (40°C, 600 MHz, dimethylformamide-*d*₇) δ 8.47–8.11 (m, 40H), 7.37–7.35 (m, 16H), 7.30 (d, 16H, *J* = 13.4 Hz), 7.07–6.99 (m, 48H), 6.79 (m, 16H), 4.56 (s, 8H), 4.49 (d, 8H, *J* = 13.4 Hz), 4.45 (d, 8H, *J* = 13.1 Hz), 4.01 (m, 8H), 3.93 (m, 8H), 3.88 (m, 8H), 3.53 (m, 8H), 3.45 (m, 8H), 3.22 (d, 8H, *J* = 13.6 Hz), 3.15 (d, 8H, *J* = 13.3 Hz), 2.25 (s, 4H), 2.23 (s, 48H), 1.89 (m, 24H), 1.42–1.25 (m, 184H), 0.92–0.88 (m, 36H). IR (thin film) 3280, 2909, 2842, 1656, 1627, 1594, 1544, 1511, 1468, and 1202 cm⁻¹. Low resolution mass spectrum (electrospray ionization; M_{avg}) calculated for C₃₄₀H₅₁₂N₄₂O₄₀ 6428, found 6428.

Freeze-fracture transmission electron microscopy samples were prepared as follows. A mixture of **1** and **2** was sonicated in CH₂Cl₂/MeOH (10:1) until homogeneous. The solvent was removed *in vacuo* and the resulting film was sonicated, with mild heating, in chloroform (≈1 g). Freeze-fracture replicas were prepared by first placing a small portion of the gel on a gold specimen carrier. The sample was cooled in a bath of liquid nitrogen (–196°C) and transferred to a Balzers BAF 400T freeze-fracture machine. The sample was fractured at –150°C and a pressure of 10⁻⁷ torr (1 torr = 133 Pa). Platinum was shadowed at a 45° angle to a depth of ≈20 Å followed by a 250- to 300-Å carbon support layer at normal incidence. The replicas were washed with CH₂Cl₂/MeOH (10:1), transferred to copper grids, and examined by using a Philips CM100 transmission electron microscope (Philips Electronic Instruments, Mahwah, NJ).

Results and Discussion

The polycaps are constructed from calixarenes that bear ureas on their upper, wider rims. The calixarenes dimerize by hydrogen bonding to form capsular host complexes ($K_d = 10^6$ – 10^8 M^{-1} ; ref. 14). When two such molecules are linked at their lower rims, as in **1** (Fig. 1*a*), the recognition elements diverge, and assembly results in a polymeric string of capsules (Fig. 1*b*). Unlike other main-chain, hydrogen-bonded polymers, the polycaps are func-

[†]R.K.C., R.C., S.L.C., and C.N. contributed equally to this work.

[¶]To whom reprint requests should be addressed. E-mail: jrebek@scripps.edu.

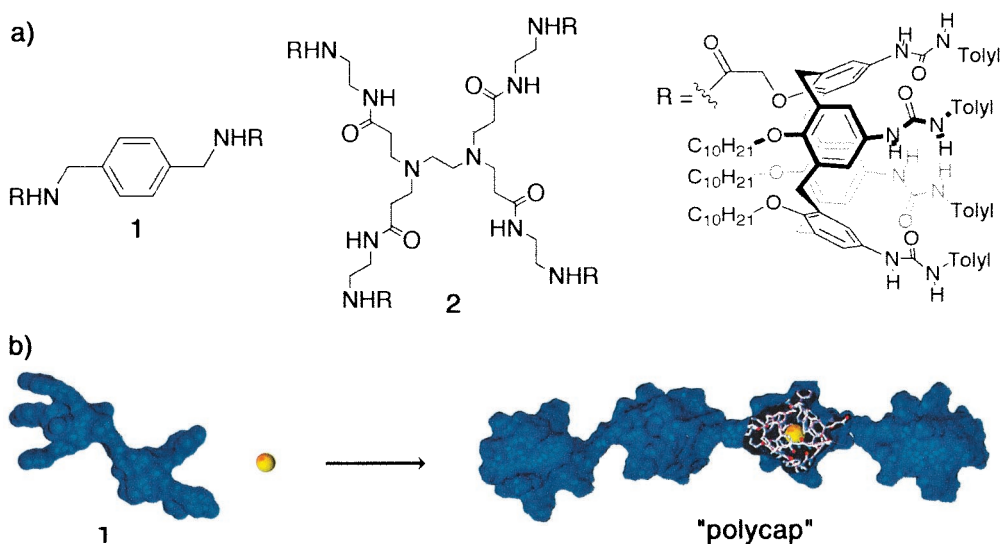


Fig. 1. (a) Polycap monomers comprising covalently linked tetraurea calixarenes **1** and **2**. (b) Dimeric assembly of **1** leads to linear, reversible polymers.

tional host units and form only when guests of proper size, shape, and chemical surface are present. For these studies, *o*-dichlorobenzene and chloroform have the dual role of solvent and guest for the calixarene capsule hosts.

The polymeric nature of **1** in *o*-dichlorobenzene is evident from its solution viscosity. As shown in Fig. 2, the viscosity increases slowly with concentration of **1** (log–log slope = 0.4) until a critical concentration of 0.6% by weight is attained, at which point the viscosity begins to rise much more significantly (slope = 3.3). This behavior is characteristic of a transition from the dilute to semidilute concentration regime (c^*), where individual polymer chains overlap and entanglements influence the observed viscoelasticity (15). The value of c^* observed here is in excellent agreement with the prediction based on independent light-scattering studies (0.6% in chloroform; A. Lomakin, G. B. Benedek, R.K.C., C.N., and J.R., personal communication), and the scaling laws (G' , $\nu \approx 1$; G'' , $\nu \approx 1.8$) agree with previous theoretical (16) and experimental (6) studies.^{||}

Unlike covalent polymers, the molecular weight distribution of the polycaps changes significantly with temperature, and this temperature dependence is reflected in their solution rheology. Typically, viscoelastic behavior from multiple temperatures may be superimposed by using a single shift factor describing the activation energy of the relaxation process (15). For the polycaps, a single parameter is not sufficient; two parameters are required, representing a concatenation of the viscoelastic activation energy and the change in molecular weight with temperature. The parameters for the TTS (time–temperature superposition) fit from 258–318 K and referenced to 298 K are: $\Delta H(\alpha) = 19.8 \text{ kcal mol}^{-1}$; $\Delta H(\beta) = 3.8 \text{ kcal mol}^{-1}$.

The reversible nature of the polymerization is also dramatic in its chemical sensitivity. Protic solvents disrupt the hydrogen-bonded assembly, and the addition of just 5% methanol turns the polymerization “off,” as shown in Fig. 3 for a 2.8% solution of **1**. With added methanol, the viscosity decreases by two orders of magnitude—from that of thin syrup back to nearly that of the pure solvent (Fig. 3). The methanol is removed easily by open heating at 50°C for a few minutes and the solution viscosity returns to its initial value, highlighting that these materials may

easily be recycled between their polymeric and monomeric forms by using extremely gentle conditions.

To what extent do the assemblies withstand physical forces, for example those imposed by shear? Viscosity decreased with increasing oscillatory shear rate (Fig. 4a), but even large, steady shear (500 s^{-1}) did not completely destroy the polymeric behavior, and instantaneous ($<1 \text{ sec}$) recovery of viscosity was observed (Fig. 4b). A more stringent test of the mechanical integrity of the polycaps is to measure physical forces such as normal stresses in solution. When polymers deform in a flow field, they may relax in directions perpendicular to the applied shear, creating normal forces in that direction (17). In noncovalent systems, an alternative response is possible; namely, the assembly can simply break into monomers if the noncovalent junction is not strong enough to withstand the forces. Despite the reversible nature of the polycap assembly, we observed normal forces in excess of 100 g ($\approx 1,000 \text{ Pa}$) from a 3.7% solution of **1** in *o*-dichlorobenzene (Fig. 5); the reversible polymerization is strong enough to withstand these disruptive conditions. This behavior is, to our knowledge, the first demonstration of significant physical integrity in hydrogen-bonded main-chain polymers in solution.

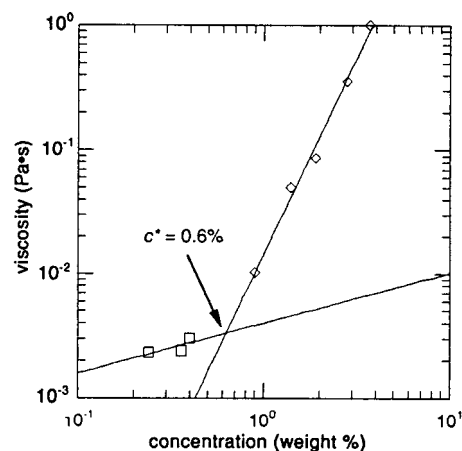


Fig. 2. Concentration dependence of the viscosity of **1** in *o*-dichlorobenzene. At 0.6% there is a transition from dilute to semidilute viscoelastic behavior (see text for details).

^{||}The c^* transition has recently been observed by Meijer and colleagues in their study of polymers formed by quadruple hydrogen-bonds. (S. H. M. Sontjens, R. P. Sijbesma, and E. W. Meijer, personal communication.)

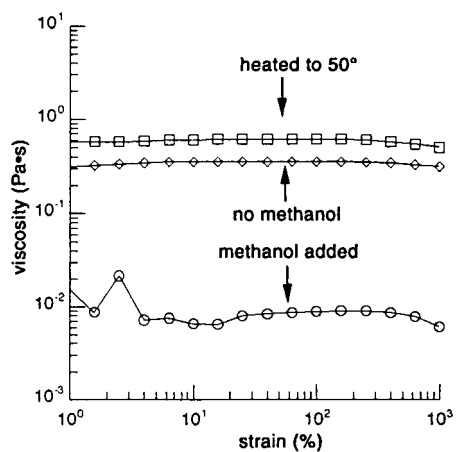


Fig. 3. The viscosity of a 2.8% solution of **1** in *o*-dichlorobenzene (\diamond) is reduced to nearly that of pure solvent on addition of 5% methanol, which reduces the polymer to monomers (\circ). The polymerization is turned back "on" by heating at 50°C for a few minutes (\square)—mild "thermosetting" conditions.

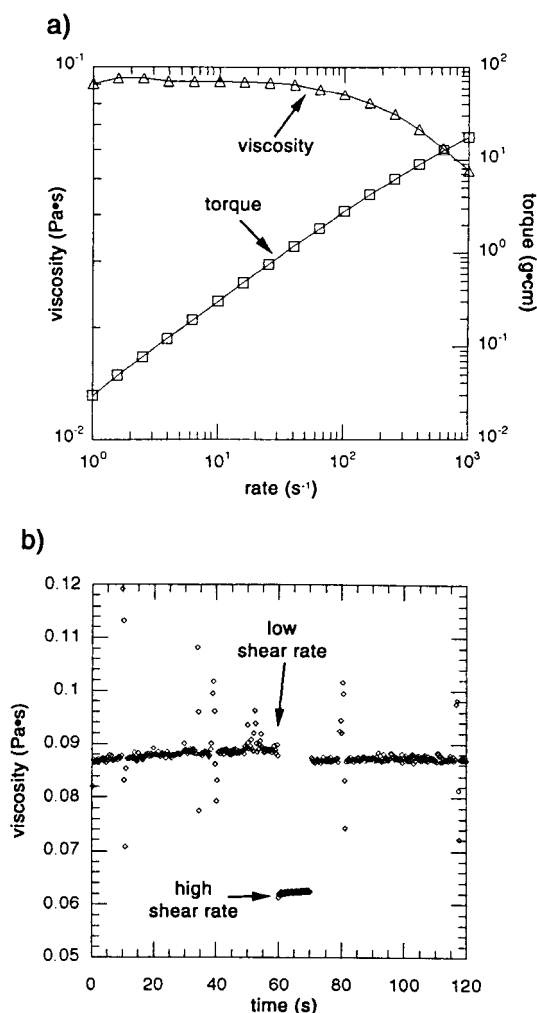


Fig. 4. (a) The viscosity of a 1.9% solution of **1** in *o*-dichlorobenzene decreases with increasing oscillatory shear. (b) When large steady shear rates of 500 s^{-1} are imposed (60–75 s) the viscosity is lowered but remains well above that of pure solvent ($\approx 10^{-3} \text{ Pa}\cdot\text{s}$) and recovers instantly when returned to an oscillatory shear rate of 10 s^{-1} .

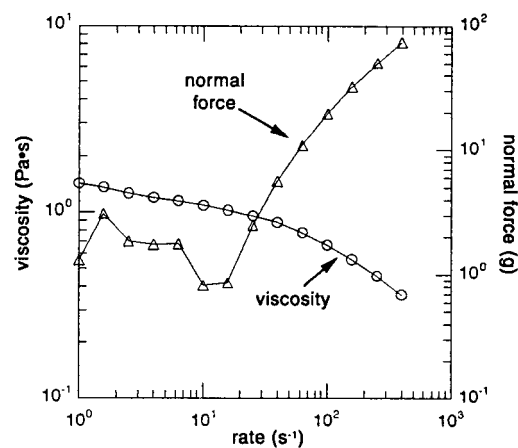


Fig. 5. Increasing normal forces with added shear in a 2.8% solution of **1** in *o*-dichlorobenzene.

The polycaps are even stronger in the solid state. Polycap **1** forms liquid crystalline phases in concentrated chloroform solutions, and fibers may be drawn from those solutions (8). Despite the crude drawing conditions, the fibers are highly ordered and have tensile strengths on the order of 10^8 Pa , or 1 g/denier as measured by the load at break (Fig. 6). By comparison, commercial nylon fibers, which are less ordered, typically have strengths of $\approx 5 \text{ g/denier}$ (18). Although covalent polymer fibers are difficult to recycle, however, the polycap fibers can easily be dissolved and redrawn on the benchtop.

Introduction of the tetravalent cross-linker **2** creates three-dimensional networks. These networks are reminiscent of physical gels, in which covalent polymers are cross-linked through weakly associative interactions (19, 20). In our system, however, the structural components are reversed: The noncovalent chains have interspersed covalent cross-links, leading to a greater number of relaxation and rearrangement pathways in the network.

As with the linear polycaps, the networks have significant mechanical integrity in *o*-dichlorobenzene solution, and the cross-linker imparts a greater elastic component to the viscoelasticity than observed in the solutions of linear polycaps. Because

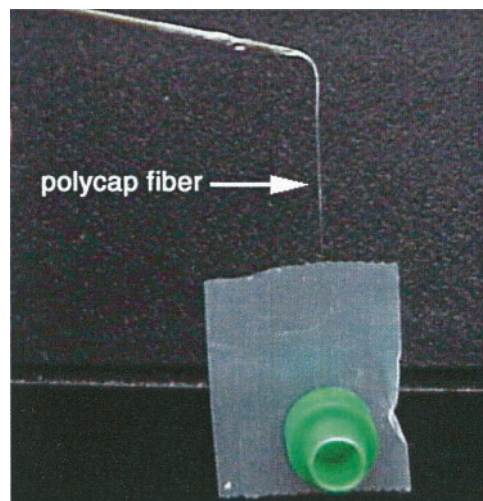


Fig. 6. A polycap fiber drawn from a liquid crystalline solution of **1** in chloroform. The fiber is connected from the tip of a needle to a piece of tape to which NMR tube caps were added until the fiber broke. The maximum supported weight was used to calculate the tenacity of the fiber (see text).

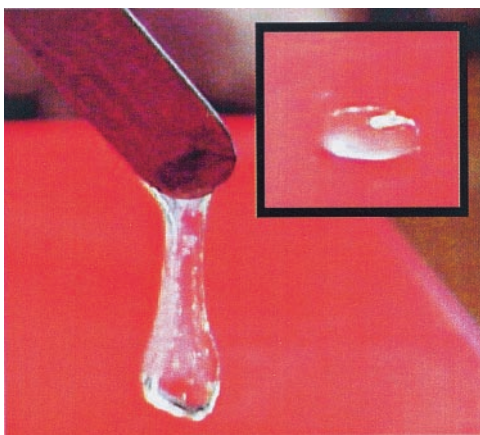


Fig. 7. Polycap gels formed of 4.5% **1** and 0.5% **2** in *o*-dichlorobenzene. The gels are elastic and resemble ring gels in their behavior. They thicken under flow and form discrete balls (*Inset*) that are rigid on short timescales but flow over longer times (several seconds), as pictured here.

of the reversible connectivity, gels formed from 4.5% **1** and 0.5% **2** are rigid on short timescales but flow on longer timescales. Like many physical gels, they are dilatant and thicken under shear to the point that a discrete ball may be formed and manipulated (Fig. 7; ref. 21). The relaxation pathway is complex and characterized by a nonexponential decay in viscoelastic properties once the shear is halted.

For a 5% solution (4:1 **1**:**2**) in chloroform, the associations are such that >99% of the polycap material should exist in effectively “infinite” networks (19). In covalent polymers, the high cross-linking density would permanently fix the structure of the network, but the polycaps retain the ability to rearrange into well ordered mesoscopic and macroscopic structures. Fig. 8 shows a transmission electron microscope surface image of the gel after a few seconds of solvent evaporation. Phase separation at the surface is apparent, and the polycap network responds to form an array of tendrils possessing a remarkably regular diameter of only a few molecules (≈ 10 nm).

In conclusion, the polycaps combine the chemical and thermal control of assembly with desirable properties such as elasticity and steric repulsion that depend on persistent polymeric structure. Although the hydrogen bonds holding the assembly together are weak enough to dissipate on relatively short timescales (from seconds to hours), the polymeric chains have measurable and meaningful mechanical integrity—even in solution. The solid-state properties of oriented fibers approach those made from traditional, covalent polymers. Finally, because assembly depends on the encapsulation of a suitable guest, the reversible polymerization can be triggered by molecular recognition, providing macroscopic structural changes in response to chemical signals.

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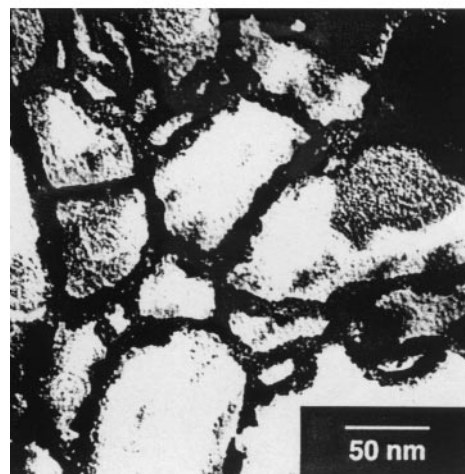
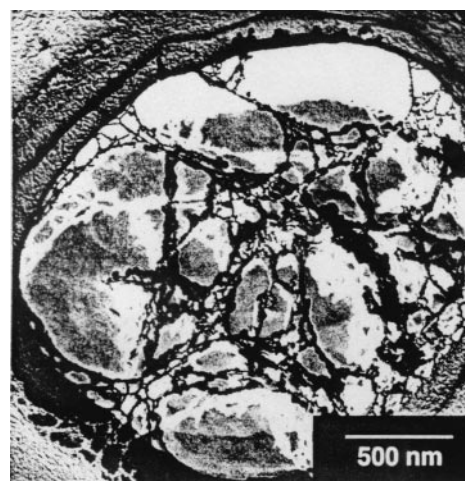


Fig. 8. Polycap networks, although extensively cross-linked, form well ordered aggregates on 10-nm length scales. The pictures show surface transmission electron microscope images from a polycap solution (4% **1**, 1% **2**) in chloroform that is frozen in liquid nitrogen after a few seconds of solvent evaporation (see text).

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