

Thermoresponsive Dynamic Covalent Single-Chain Polymer Nanoparticles Reversibly Transform into a Hydrogel**

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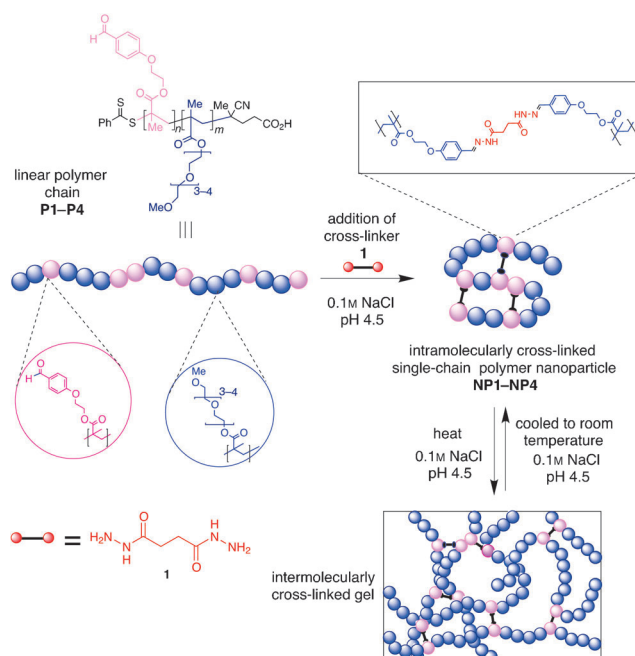
Emerging applications in materials, biomedical, and nano sciences demand new “intelligent” polymeric materials, which possess the capacity to adapt or undergo a macroscopic response to changes in their environments.^[1] In this regard, there exist already a number^[2] of conventional stimuli-responsive polymers that can respond to input stimuli, such as changes in temperature or pH, by undergoing a reversible phase transition. Although well-studied and exploited,^[3] these polymers still present a rather limited palette of structure, property, and responsiveness, and chemists have been actively researching alternative means to endow polymers with the virtues of adaptive and responsive features.

An approach of increasing importance towards intelligent polymeric materials is the utilization of so-called dynamic covalent bonds (DCBs),^[4] which, when judiciously placed either into or between polymer chains, present key features that can be exploited to endow polymeric species with responsive and adaptive properties.^[5] A key feature of DCBs is their capacity to undergo component exchange processes involving the exchange of one reaction partner for another (for example, the reaction of an imine with an amine to produce a different imine and amine), which can present polymeric species with opportunities to adapt their constitutions by reshuffling components, and thus change their properties.^[6] As these exchange process usually require the help of a suitable catalyst to aid kinetics, the option exists to halt these processes and kinetically fix the products by quenching the catalyst, an option not available with non-covalent bonds. A further advantage of employing DCBs within responsive and adaptive polymeric materials is that the strength of the covalent bond ensures the resultant materials possess chemical robustness, an important issue when considering possible applications.

The development of increasingly sophisticated intelligent polymeric species will require the combination of different stimuli-responsive elements. We became intrigued by the possibility of developing such materials through the combination of conventional polymers that adapt to stimuli with a physical response,^[7] and DCBs able to continuously adjust their constitution in the presence of a suitable catalyst. Such

a union of physical and dynamic covalent interactions has led to complex molecular architectures when combined with small molecules,^[8] but has seen little exploitation on macro-molecular scales. Herein, we demonstrate how the capacity of DCBs to undergo component exchange can, in combination with conventional thermo-responsive polymers, be harnessed to prepare water-soluble dynamic covalent polymeric nanoparticles that can undergo a remarkable reversible transformation into a macroscopic hydrogel network.^[9]

We have produced single-chain polymer nanoparticles (SCPNs) (**NP1–NP4**, Scheme 1) based upon previously described^[10] water-soluble OEGMA₃₀₀ copolymers (**P1–P4**,



Scheme 1. Conjugation of polymer chains **P1–P4** with **1** to form intramolecularly cross-linked SCPNs **NP1–NP4**, and their subsequent reversible transformation into an intermolecularly cross-linked hydrogel.

Table 1) that feature reactive aromatic aldehyde functions, which serve as handles for the formation of acyl hydrazone cross-links. These copolymers are prepared by the copolymerization of the monomers OEGMA₃₀₀ and *p*-(2-methacryloxyethoxy)benzaldehyde (MAEBA; see the Supporting Information), and are of similar molecular weights (*M_w* 37–61 kDa), but differ in their densities of displayed aldehyde functions, as measured by the molar weight percentage of the aldehyde-containing monomer within the copolymer chains.

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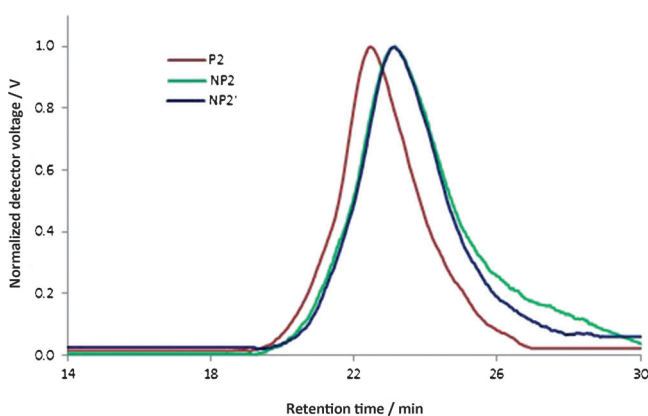
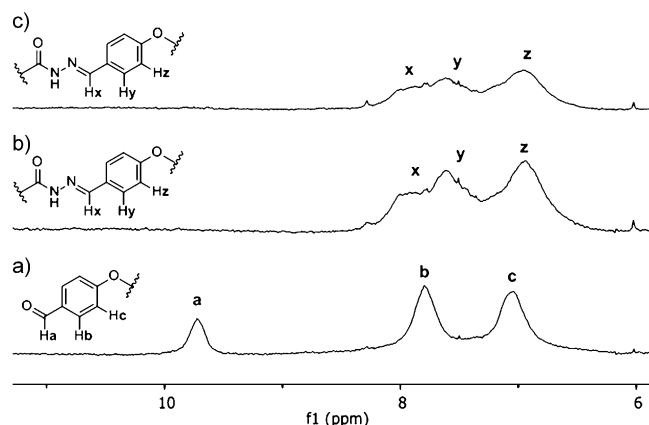
Table 1: Characterization of polymers **P1–P4** and nanoparticles **NP1–NP4** and **NP1'–NP4'**.

Polymer	MW _{ACM} [%] ^[a]	M _n [Da] ^[b,j]	M _w [Da] ^[c,j]	PDI ^[d,j]	LCST [°C] ^[e,k]	Δ _{LCST} [°C] ^[f,k]	T _{GEL} [°C] ^[g]	t _{SOL} [days] ^[h]	t _{SCPN} [days] ^[i]
P1	20.5	33 800	45 300	1.34	32.0	12.5	48.5	20.0	> 60
P2	16.5	47 100	60 900	1.30	37.5	10.0	52.5	5.0	30
P3	9.0	31 600	40 800	1.26	49.0	3.0	57.0	2.0	7
P4	4.5	29 700	37 100	1.21	52.0	2.5	66.0	0.5	3

[a] Molar weight % of aldehyde-containing monomer. [b] Number-average molecular weight. [c] Weight-average molecular weight. [d] Polydispersity index. [e] Lower critical solution temperature. [f] Change in LCST upon formation of SCPNs **NP1–NP4**. [g] Gelation temperature. [h] Time taken for re-dissolution of gel. [i] Time taken for complete re-equilibration to SCPNs **NP1'–NP4'**. [j] As determined by GPC in DMF containing LiBr (1 g L⁻¹; 0.6 mL min⁻¹) calibrated against near monodisperse poly(methyl methacrylate) standards. [k] Determined by turbidimetric analysis in AcOH/NH₄OAc buffer (pH 4.5). LCST = temperature at 50% transmittance.

This series of copolymers allows us to explore the effects of cross-linking density upon the reversible cross-linking process. All copolymers possess sufficiently low polydispersities (PDI < 1.4) for their use in this study. These linear copolymers display thermoresponsive behavior, and reversibly precipitate from aqueous solution when heated above their lower critical solution temperatures (LCSTs; Table 1), a quality which provides a thermoresponsive feature to the resultant SCPNs. Acylhydrazone bonds are well-known^[11] to undergo exchange processes at acidic pH (with optimum kinetics at pH 4.5), but are kinetically fixed at pH 7. This feature endows the nanoparticles with a binary response to pH that can be usefully combined with the thermoresponsive nature of the copolymers.

SCPNS **NP1–NP4** were prepared (Scheme 1) by the addition of low-molecular-weight dihydrazide **1** to a solution of copolymers **P1–P4** (0.1–1 wt %) in AcOH/AcONH₄ buffer (0.1M, pH 4.5), thus causing intramolecular cross-linking through the formation of dynamic covalent acylhydrazone bonds. The cross-linking process was followed by gel permeation chromatography (GPC), which revealed an increased retention time relative to linear copolymers (Figure 1; see also the Supporting Information, Figures S8–S11), an observation that is consistent with other work^[12] in the field of SCPNs and indicates the collapse of the polymer chain along with concomitant reduction in its hydrodynamic volume. Further evidence for the formation of SCPNs was seen by comparing the ¹H NMR spectra of the linear copolymer and


Figure 1. Differential refractive index gel permeation chromatography (GPC) traces in DMF (0.6 mL min⁻¹, containing LiBr 1 g L⁻¹).

Figure 2. Partial ¹H NMR spectra (400 MHz, D₂O, pH 4.5, 20 °C) of a) copolymer **P2**, b) SCPN **NP2** and c) SCPN **NP2'**.

its corresponding nanoparticles (Figures 2 and S4–S7). For example, when **P2** was reacted with dihydrazide **1**, a broadening of signals associated with the aromatic protons ($\delta = 7.1$, 7.8 ppm) within the polymer scaffold was observed, along with the disappearance of the signal corresponding to the aromatic aldehyde ($\delta = 9.8$ ppm) and the appearance of a signal ($\delta = 8.1$ ppm, labeled “x” in Figure 2) corresponding to hydrazone formation. Estimation of the LCSTs of **NP1–NP4** was performed by cloud point determination at pH 4.5. In all cases, the LCSTs of the SCPNs were observed (Table 1) to be higher than that of their linear precursors, presumably on account of the relative hydrophilicity of cross-linker **1**.

Constitutional reorganization of the nanoparticles into a hydrogel network was triggered simply by raising the temperature of the nanoparticle solutions above the estimated LCSTs at pH 4.5. As expected, a white precipitate was seen to form in all cases. Upon raising the solution temperature, no change was seen until the gelation temperature^[13] was reached, whereupon after 5 min the precipitate was seen to form an opaque solid material. Vial inversion tests indicated that this material possessed hydrogel-like characteristics, which suggests the reorganization of the polymer chains within the nanoparticles from intra- to intermolecularly cross-linked. When samples of the hydrogel (75 mg) derived from **NP1** were transferred to a selection of organic solvents (1 mL each of MeOH, CH₂Cl₂, DMSO, and DMF) dissolution was not observed after more than 40 days, compared with full

dissolution of **P1** in <10 min in these same solvents. This simple chemical test further suggests the existence of chemical cross-links within the gel material, and implies that the gel does not exist as a physical aggregation of SCPNs.

We hypothesize that this constitutional reorganization of SCPNs into a hydrogel arises on account of the combination of supramolecular and molecular dynamics. When heated above their LCSTs the nanoparticles become hydrophobic and aggregate, a process occurring on the supramolecular level. The highly localized nanoparticle concentration then facilitates intermolecular reorganization of the acylhydrazone crosslinks at the molecular level, with intramolecular cross-links cleaved to create a significant number of isoenergetic interchain cross-links, thus leading to the formation of a macroscopic hydrogel.

The reverse transformation of the hydrogel network back into SCPNs (**NP1**–**NP4**)^[14] was triggered by simply cooling the sample to room temperature, with the hydrogel material slowly redissolving and returning to the sol state after 0.5–20 days (Table 1). The increased time taken for the reverse transformation indicates that the de-cross-linking of polymer chains is a considerably slower process than the forward gelation process. We hypothesize that this slowdown is on account of the multivalency of the inter-polymer bonding, whereby the high cross-linking densities within the hydrogel inhibit separation and solvation of individual polymer chains, as all intermolecular cross-links must be broken before this separation is possible. This hypothesis is supported by the observation that, as the level of cross-linking decreases from **NP1**–**NP4**, so too does the time taken for the resultant hydrogels to re-dissolve upon cooling (Table 1). To investigate the reversibility of this gelation process in more detail, GPC traces, ¹H NMR spectra, and dynamic light scattering (DLS) data were gathered from the aqueous SCPN solutions formed after redissolution of hydrogel. The GPC chromatograms showed highly polydisperse nanoparticles (Figure S12) to be present after initial re-dissolution, which slowly re-equilibrated to afford GPC traces (Figures 2 and S8–S11) very similar to that of the starting SCPNs **NP1**–**NP4**. DLS analysis of **NP1**–**NP4** showed (Figures S14–S16) a single monomodal particle distribution identical to that of the starting SCPNs **NP1**–**NP4**. Similarly, the ¹H NMR spectra of **NP1**–**NP4** were found to be similar to those of the initially-formed SCPN **NP1**–**NP4** (Figures S4–S7). Taken together, all of these experiments indicate that upon cooling the hydrogel reorganizes back into a solution of SCPNs.

By kinetically fixing the dynamic acylhydrazone exchange, it was possible to trap the polymer chains in either their SCPN or hydrogel forms. For example, heating a solution of **NP3** to 65°C at pH 7, at which point the acylhydrazone bonds are kinetically fixed, did not result in hydrogel formation and the SCPNs merely formed a white precipitate that readily redissolved upon cooling to room temperature. Furthermore, when a solution of **NP3** at pH 4.5 was heated at 53°C to form the hydrogel and then the pH of the supernatant adjusted to 7, the gel did not redissolve for several months when cooled back to room temperature. Readjustment of the pH to 4.5 resulted in the gel dissolving after one day. Maintaining the pH at 7 has, in effect, frozen

the molecular dynamics of the SCPNs and thus prevented the gelation process from occurring. To further highlight that dynamic covalent cross-links are required to facilitate gel formation, the acyl hydrazone bonds within **NP1** were reduced by reaction with NaBH₃CN to form non-reversible covalent amine cross-links. When these nanoparticles were raised above their LCST, the resulting precipitate quickly redissolved when the temperature was lowered back below the LCST, a result which indicates that nanoparticles absent in dynamic covalent cross-links cannot form hydrogels.

In summary, we have reported that SCPNs are able to reversibly undergo a transition into a chemically cross-linked hydrogel upon raising the temperature of their aqueous solutions above their LCSTs at mildly acidic pH, a process which is facilitated both by the thermoresponsive nature of the polymer chains and the capacity of dynamic covalent acylhydrazone bonds to undergo component exchange processes. This synergy of conventional stimuli-responsive polymer with DCBs results in a polymeric material possessing unique adaptive features. Such triggered gel formation requires the simultaneous application of both low pH and temperature, and the polymeric species described here are thus also a rare example of a material which requires the simultaneous application of two orthogonal stimuli to trigger response.^[15] The ability to obtain a response by using a combination of stimuli applied simultaneously could lead to greater specificity regarding where and when events are triggered, a feature which would be highly advantageous in fields such as drug delivery, and we speculate that such materials will become increasing topics of future interest.

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