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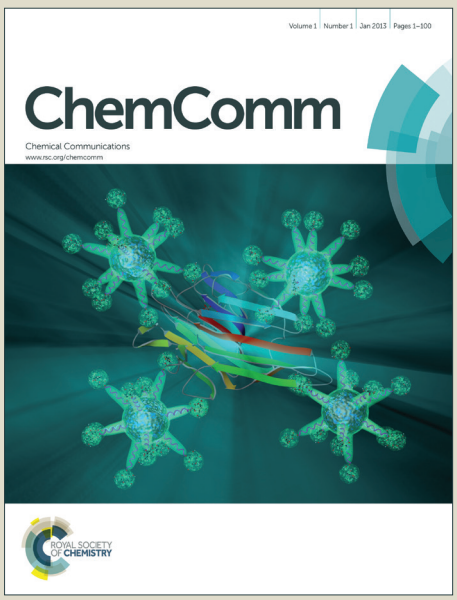

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COMMUNICATION

pH-Responsive Single-Chain Polymer Nanoparticles Utilising Dynamic Covalent Enamine Bonds

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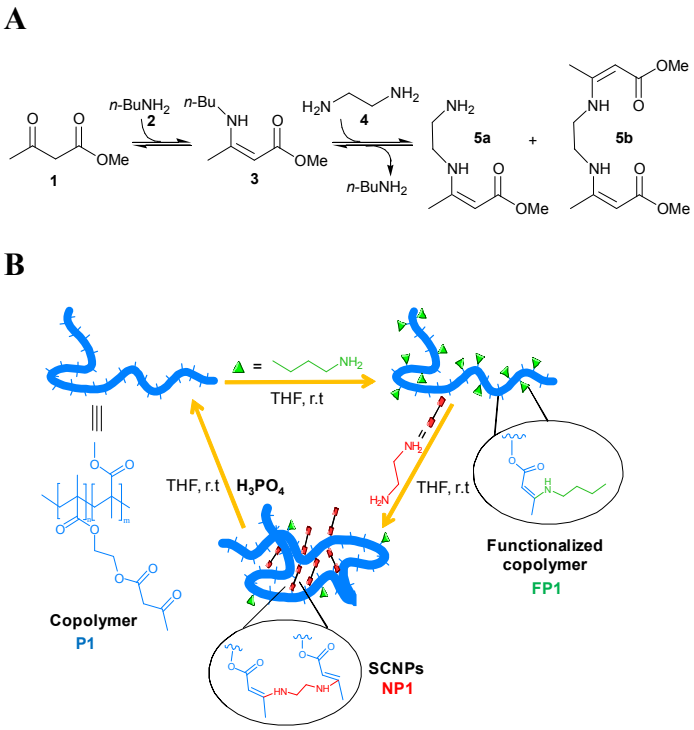
Structurally dynamic single-chain polymer nanoparticles that can reversibly undergo a coil to particle transition via formation and cleavage of intramolecular dynamic enamine cross-links are reported.

Interest in dynamic covalent bonds (DCB)[1-3] that can be reversibly cleaved and reformed upon exposure to an external stimuli has grown significantly in recent years. The incorporation of DCBs into polymeric systems (*e.g.,* micelles, films, fibers) allows access to dynamic structures which can show macroscopic responses to a range of external stimuli (*e.g.,* temperature, pH, redox processes).[4-5] To date, however, only a relatively small number of dynamic covalent bonds (*e.g.* imine, disulfide and boronic esters) have been utilized in the construction of dynamic polymeric materials[6], and to expand further their utilisation there exists a need for a greater selection of dynamic bonds. The enamine bond[7,8] belongs to the family of imine and hydrazone DCBs[4], however, to the best of our knowledge, it has not yet been employed for the development of structurally dynamic polymeric systems. The enamine bond can undergo different dynamic processes, displaying i) the possibility to reversibly cleave and reform under appropriate conditions, and ii) the ability to undergo component exchange. Both of these processes operate under thermodynamic control. A particular advantage of enamine DCBs is that a carbonyl reaction partner can be readily incorporated into polymer chains as the monomer 2-(acetoacetoxy)ethyl methacrylate—which displays a suitably reactive carbonyl function—is commercially available. This availability circumvents the need to synthesize designer monomers possessing reactive carbonyl groups, consequently making the incorporation of DCBs within dynamic polymer systems less time- and labour-consuming.

To demonstrate the utility of dynamic covalent enamine bonds in the preparation of responsive polymer materials, we report here their utilisation to prepare pH-responsive single-chain polymer nanoparticles (SCNPs) (Scheme 1). SCNPs constitute a class of polymeric materials of intense current interest in which single linear polymer chains are intramolecularly cross-linked into ultra-small soft nano-objects[9-10] which show intriguing nanoscale properties and are of great interest for numerous potential applications[10,11] including their use as rheology-improving agents for melts of thermoplastics, elastomeric polymers, nanocomposites and paints. The introduction of responsiveness into SCNPs through the use of DCBs[12-13] provides soft nano-objects with structurally dynamic properties which increases their potential utilisation as so-called 'smart' materials which may find application in nanomedicine, catalysis and the development of dynamic combinatorial libraries.[14,15]

We firstly investigated the dynamic nature of enamine bonds with model low molecular weight compounds. Equimolar amounts of methyl acetoacetate (**1**) and butylamine (**2**) were reacted to form the *mono*-enamine **3**. After 24 h ^{1}H NMR spectroscopy revealed signals associated with the enamine bond



Scheme 1. A) The formation of an enamine **3** from the condensation of methyl acetoacetate (**1**) and *n*-butylamine (**2**), and its component exchange reaction with ethylenediamine (**4**) to form a *bis*-enamine (**5**). B) Schematic illustration of assembly / disassembly of single-chain polymer nanoparticles (SCNPs) by means of dynamic covalent enamine bonds.

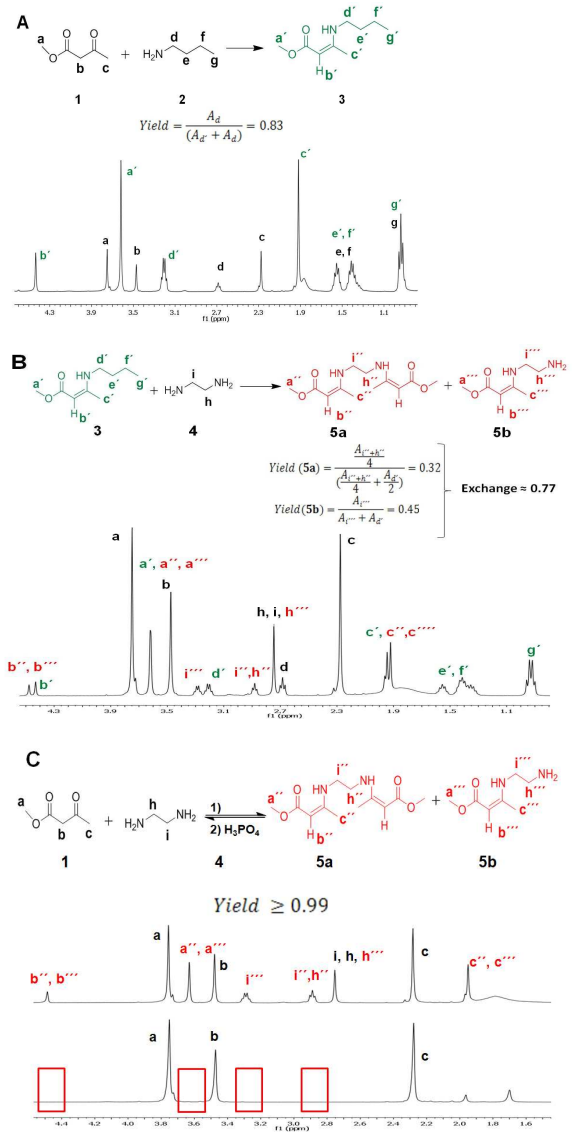


Fig. 1. Low molecular weight compounds were selected for the investigation of enamine dynamic covalent bonds: A) Upon mixing methyl acetoacetate (**1**) and butylamine (**2**) for 24 h, the ^{1}H NMR spectrum shows the characteristics bands of enamine formation at 3.2 ppm and 4.5 ppm (see green letters) for the new compound (E,Z)-methyl 3-(butylamino)but-2-enoate (**3**). B) After addition of ethylenediamine (**4**) to the reaction medium, new bands (see red letters) associated to protons from compounds **5a** and **5b** appeared in the ^{1}H NMR spectrum upon enamine exchange reactions. C) Illustration of acid-triggered enamine disruption upon addition of deuterated phosphoric acid (bottom spectrum) to compounds **5a** and **5b** (upper spectrum).

of **3** that were clearly visible at 3.2 and 4.5 ppm (Fig. 1A). The conversion of **1** and **2** into **3** was estimated to be 83%, indicating the equilibrium lies on the side of the enamine. Component exchange of the enamine bond was induced by the addition of ethylenediamine (**4**) (0.5 equivalents with respect to **1**) to the reaction mixture, driving the formation of the *mono-* and *bis-*enamines (**5a, 5b**). Examination of the ^{1}H NMR spectrum revealed the appearance of new enamine signals at 2.8, 3.30 and 4.55 ppm arising from compounds **5a** and **5b** (Fig. 1B). Additionally, we performed ^{1}H NMR experiments to investigate acid-triggered enamine bond rupture. Upon addition of phosphoric acid, ^{1}H NMR spectroscopy indicated a quantitative hydrolysis of enamine bonds occurred (Fig. 1C). Taken together, these preliminary experiments demonstrate that enamines can form spontaneously, undergo component exchange processes, and quantitatively hydrolyse back to starting materials upon the addition of an acid.

Table1. Characteristics of copolymers **P1-P4** and SCNPs **NP1-NP4**

Sample	M_w (g/mol)[a]	M_w/M_n	AEMA (mol%)	D_h (nm)[b]
P1	30 900	1.05	31	7.8
P2	38 000	1.06	30	8.5
P3	53 500	1.05	26	10.6
P4	309 400	1.3	30	27.7
NP1	29 800	1.04	31	5.3
NP2	39 100	1.04	30	5.7
NP3	52 700	1.05	26	6.8
NP4	289 900	1.2	30	14.1

[a]Actual weight average molecular weight as determined by combined SEC/MALLS measurements. [b]Hydrodynamic diameter, D_h, as determined by DLS.

We next explored the use of enamine DCBs for the construction of responsive SCNPs (Scheme 1B). Four poly(methyl methacrylate-*co*-(2-acetoacetoxy)ethyl methacrylate) (P(MMA-co-AEMA)) random copolymers (**P1-P4**) featuring reactive *β*-ketoester functional groups of different molecular weights (Table 1) were synthesized through reversible addition-fragmentation chain-transfer (RAFT) polymerization.[16] Copolymers **P1-P4** were functionalized *via* enamine formation with the monofunctional amine butylamine (**2**) in THF solution (see SI) to afford the functionalized linear polymers **FP1-FP4**. Fourier transform infrared (FTIR) spectroscopy (Fig. 2A) confirmed successful enamine bond formation, as evidenced by the significant narrowing of the C=O stretch associated with the AEMA units and the appearance of new infrared vibration bands at 1650 cm^{-1} and 1605 cm^{-1} which we assign to stretching vibrations of enamine bonds.

The functionalized linear polymers **FP1-FP4** were then collapsed progressively to SCNPs through exchange reactions with ethylenediamine (**4**), which takes the role of an intrachain cross-linker (Scheme 1B). SCNP synthesis was performed in THF at a concentration of 1 mg/ml of copolymer using equimolar concentrations of *β*-ketoester and amine functional groups. The chain collapse process was followed by size exclusion chromatography (SEC) (Fig. 2B), which revealed a significant increase in retention times (t_R) relative to **FP1-FP4**, observations consistent with a reduction in hydrodynamic sizes[17] and which suggest strongly that intramolecular collapse induced by cross-linking has occurred *via* exchange reactions of the pendant enamines in **FP1-FP4** with the bifunctional ethylenediamine units. In order to quantify the changes in hydro dynamic sizes between **P1-P4** and their corresponding SCNPs **NP1-NP4**, dynamic light scattering (DLS) measurements were performed. As a representative example, the size of copolymer **P1** reduced from D_h = 7.8 nm to D_h = 5.3 nm upon SCNP formation (Table 1 and Fig. S2). Further evidence supporting SCNP formation was obtained by ^{1}H NMR spectroscopy. Comparison of the ^{1}H NMR spectrum of the P(MMA-co-AEMA) random copolymer **P1** (Fig. 3A) with that of its corresponding SCNP **NP1** (Fig. 3B) reveals significant peak broadening upon intramolecular cross-linking. Insets in Figure 3B show clear band broadening in signals arising

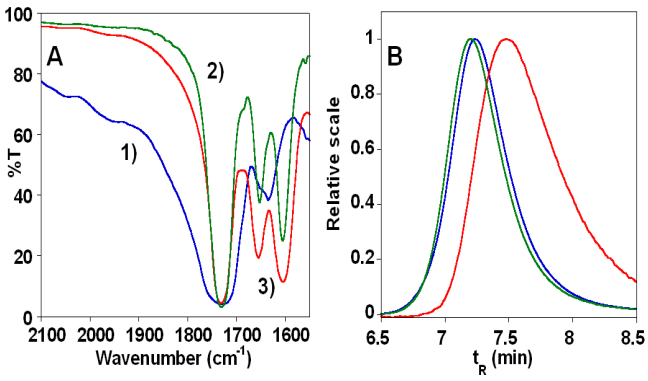


Fig. 2 Single-chain polymer nanoparticle (SCNP) construction *via* enamine dynamic covalent bonds (DCBs): A) FTIR spectra corresponding to: 1) P(MMA-*co*-AEMA) copolymer **P1** (blue line), 2) butylamine-functionalized copolymer **FP1** (green line), and 3) SCNPs **NP1** synthesized through dynamic butylamine / ethylenediamine exchange (red line). B) Illustration of SEC traces for **P1** (blue line), **FP1** (green line), and **NP1** (red line). For the sake of clarity only the data associated with **P1** are presented here.

from –CH= protons of enamine groups (4.3 ppm, broad signal) and -CH_3 protons of the main-chain methyl groups (0.7–1.2 ppm, broad signal) of **NP1**. For SCNPs, signal broadening in the ^{1}H NMR spectrum can be attributed to the restricted mobility of some of the SCNP protons as a consequence of the progressive cross-linking and is a well-documented signature of SCNPs formation.[18,19] Taken together, these experiments demonstrate that enamine exchange processes can facilitate successfully the formation of SCNPs.

To demonstrate the pH-responsiveness of these SCNPs, phosphoric acid (H_3PO_4) was added to the reaction medium to cause acid-triggered disassembly of the SCNPs. Upon acid treatment of **NP1** a decrease in retention time in the SEC trace was observed (Fig. 4) suggesting an increase in the hydrodynamic size. After treatment for 24 h with excess H_3PO_4 the collapsed copolymer chains re-expand to their original hydrodynamic size (based on SEC retention time of the original copolymer **P1**) regardless of the amount of cross-linker originally added, confirming a highly efficient acid-triggered SCNP disassembly process. In fact, SEC traces of the original P(MMA-co-AEMA) copolymer **P1** and the disassembled SCNPs, denoted as **P1´**, were found to be nearly identical (Fig. 4). Upon SCNP disassembly the specific enamine infrared vibration bands totally disappeared (Fig. S3) and the ^{1}H NMR spectrum of **P1´** (Fig. 3C) was found to be very similar to that of the initial copolymer **P1** (Fig. 3A). The resulting copolymer **P1´** was successfully isolated by precipitation in water, drying under vacuum and redissolved in THF. To further demonstrate the possibilities of enamine DCBs, reorganization of **P1´** into SCNP, denoted as **NP1´**, was triggered *directly* by addition of cross-linker ethylenediamine (**4**) (0.5 equivalents with respect to AEMA groups) to **P1´**. As expected, a significant shift in the SEC trace towards longer retention time was clearly observed, again indicating a reduction in hydrodynamic size upon the reformation of the enamine bonds and the concomitant intrachain cross-linking. Fig. 4 shows overlays of the chromatograms for the original SCNPs **NP1** and the reformed SCNPs **NP1´**, showing very similar SEC traces. Moreover, both exchange reactions between alkyl monoamine/alkyl diamine and acid-triggered disassembly of the

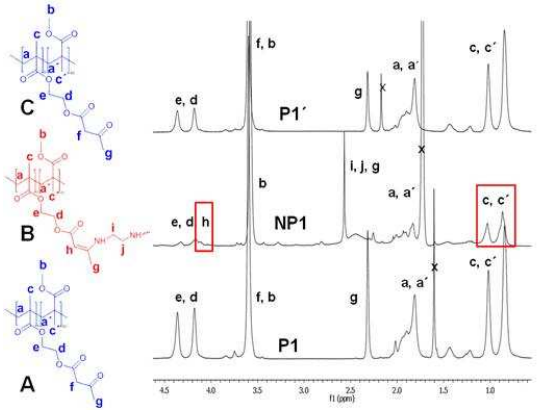


Fig. 3 ^{1}H NMR spectra corresponding to: A) P(MMA-co-AEMA) copolymer **P1**. B) SCNPs **NP1** synthesized through dynamic alkyl amine / alkyl diamine exchange. C) Disassembled SCNPs, denoted as **P1´**, after addition of an excess of H_3PO_4 with respect to the amount of enamine DCBs in **NP1**.

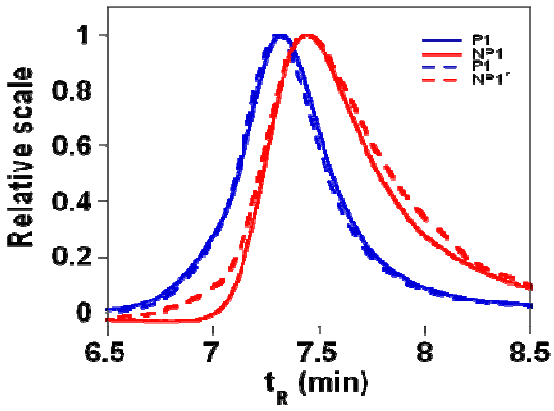


Fig. 4 Illustration of SCNP disassembly and reassembly: SEC trace of **NP1** (continuous red line), SEC trace of **NP1** after H_3PO_4 addition (**P1´**) (broken blue line) and SEC trace of the re-formed SCNP **NP1´** synthesized directly from **P1´** and **4** (broken red line). SEC trace of **P1** (continuous blue line) is also included for comparison.

SCNPs can be easily performed in a one-pot fashion as illustrated in Fig. S4. Preliminary experiments showed a disassembly degree of 100 % at pH = 5 in 16 h of reaction time, whereas at pH = 6, the disassembly degree amounted to 40 % after 24 h (see Table S2).

In conclusion, we have demonstrated the facile one-pot reversible conversion of linear polymer chains into pH-responsive SCNPs by means of enamine DCBs. This proof of concept paves the way to the use of enamine DCBs for the construction of other responsive, structurally dynamic polymeric systems beyond SCNPs. Due to the availability and important antitumor role of some natural occurring diamines (*e.g.,* norspermidine), the use of enamine DCBs for the development of responsive therapeutic nanocarriers and/or innovative sensor nanodevices is envisioned. This work will also renew interest in enamines as DCBs, which have been surprisingly overlooked as a dynamic covalent bond. Our preliminary results suggest that this "*forgotten*" DCB will become a valuable addition to the growing 'toolbox' of dynamic covalent chemistry.

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Notes and references

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