Supplementary Information

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

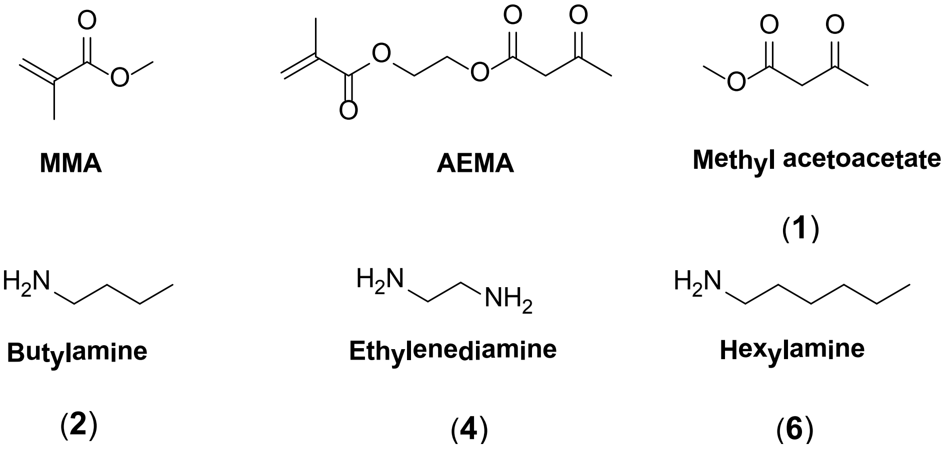
Ana Sanchez-Sanchez, David A. Fulton* and José A. Pomposo*

***E.mails: d.a.fulton@ncl.ac.uk and Josetxo.pomposo@ehu.es**

S1. Materials

Methyl methacrylate (**MMA**) (99%), (2-acetoacetoxy)ethyl methacrylate (**AEMA**) (95%), 2,2-azobis(2-methylpropionitrile) (≥98%), ethylenediamine (≥99%), hexylamine (≥99.5 %), butylamine (99.5%), phosphoric acid (ACS reagent, ≥85 wt.% in H_2O), methyl acetoacetate (99%), ethyl acetate (anhydrous, 99.8 %), diethyl ether (ACS reagent, anhydrous, >99.0%), hexane (ACS reagent, ≥99.5%), deuterated chloroform (99.96 atom % D, containing 0.03 % (v/v) tetramethylsilane, TMS), deuterated tetrahydrofuran (99.5 atom % D) were purchased from Aldrich and used, unless specified, as received. 2-Cyanoprop-2-yl-dithiobenzoate (CPDB) (≥97%) was purchased from Strem Chemicals. Methanol (synthesis grade) and tetrahydrofuran (HPLC grade) were purchased from Scharlab. MMA was purified by distillation before use. AEMA was purified by passing through basic alumina.

Scheme S1. Chemical structures of some of the compounds used in this work.



S2. Methods

Model reactions with model compounds: In a typical procedure, methyl acetoacetate **(1)** (15 mg, 0.3 mmol) and butylamine **(2)** (9.5 mg, 0.3 mmol) were dissolved in deuterated cloroform (1 ml). The solution was left to stir for 24 h at r.t. After 24 h the ^{1}H NMR spectroscopy revealed signals associated to the enamine bond of (*E,Z*)-methyl 3-(butylamino)but-2-enoate (**3**). Component exchange was induced by the addition of ethylenediamine (**4**) (3.9 mg, 0.065 mmol) to the reaction mixture. The ^{1}H NMR spectrum revealed the formation of mono- and bis-enamines.

*Synthesis of P(MMA-co-AEMA) copolymers **P1-P4***: In a typical procedure, MMA (1 ml, 9.4 mmol), AEMA (0.6 ml, 3.1 mmol), 2-cyanoprop-2-yl-dithiobenzoate (7.2 mg, 3.25×10^{-2} mmol) and 2,2-azobis(2-methylpropionitrile) (0.8 mg, 4.9×10^{-3} mmol) were dissolved in ethyl acetate (3.2 ml). The reaction mixture was degassed by passing argon for 15 min. The copolymerization reaction was carried out at 65ºC for 18 h. The resulting copolymer **P1** was isolated by precipitation in methanol and further drying (68% yield).

*Synthesis of alkyl amine-funcionalized P(MMA-co-AEMA) copolymers **FP1-FP4**:* In a typical procedure, P(MMA-co-AEMA) copolymer **P1** (100 mg, 0.3 mmol) and alkyl amine (butylamine, **2**: 0.04 ml, 0.6 mmol or hexylamine, **6**: 0.06 ml, 0.6 mmol) were dissolved in THF (30 ml). The solution was left to stir for 24 h, concentrated under reduced pressure and precipitated by its drop-wise addition to hexane and then isolated by filtration. The main characteristics of functionalized copolymers **FP1-FP4** are summarized in Table S1.

Table S1. Characteristics of alkyl amine-functionalized copolymers **FP1-FP4**

Polymer	M_w (g/mol)[a]	M_w/M_n	Functionalization[b]	Yield (%)[c]
FP1	32 400	1.05	90	70
FP2	41 800	1.05	92	69
FP3	53 300	1.06	91	75
FP4	-	-	-	-

[a]Actual weight average molecular weight as determined by combined SEC/MALLS measurements (dn/dc= 0.083).[b]Content of alkyl amine-functionalizated AEMA units (mol%) in the copolymer as determined by ^{1}H NMR spectroscopy. [c]Calculated as grams of functionalized copolymer /initial grams of copolymer × 100.

Synthesis of SCNPs ***NP1-NP4****:* In a typical reaction, alkyl amine-funcionalized copolymer **FP1** (50 mg, 0.11 mmol) and cross-linker (ethylenediamine, **4**: 3.4 mg, 0.055 mmol) were dissolved in THF (50 ml) at room temperature. The progressive folding/collapse process was followed through simultaneous SEC/MALLS measurements. After reaction completion (24 h), the mixture was concentrated and SCNPs **NP1** were isolated by precipitation in diethyl ether and further drying (79% yield).

Acid-triggered disassembly of SCNPs ***NP1-NP4****:* In a typical reaction, **NP1** (50 mg, 0.11 mmol) and phosphoric acid (H_3PO_4, excess with respect to AEMA content in **P1**) were dissolved in THF (50 ml) at room temperature. The unfolding process was followed through simultaneous SEC/MALLS measurements. After reaction completion (24 h), the mixture was concentrated and copolymer **P1´** was isolated by precipitation in distillated water (85% yield).

Re-assembly of SCNP ***NP1´***: In a typical reaction, **P1´** (50 mg, 0.11 mmol) and cross-linker (ethylenediamine, **4**: 3.4 mg, 0.055 mmol) were dissolved in THF (50 ml) at room temperature. The re-formation process to SCNPs was followed through simultaneous SEC/MALLS measurements. After reaction completion (24 h), the solution was concentrated and the re-formed SCNPs ***NP1´*** were isolated by precipitation in diethyl ether (78% yield).

S3. Characterization Techniques

Nuclear Magnetic Resonance (NMR) spectroscopy: ^{1}H NMR spectra were recorded at room temperature on a Bruker spectrometer operating at 400 MHz.

Size Exclusion Chromatography / Static Light Scattering (SEC/MALLS): SEC/MALLS measurements were performed at 30 ºC on an Agilent 1200 system equipped with PLgel 5µm Guard and PLgel 5µm MIXED-C columns, a differential refractive index (RI) detector (Optilab Rex, Wyatt) and a SLS detector (Minidawn Treos, Wyatt). Data analysis was performed with ASTRA Software from Wyatt. THF was used as eluent at a flow rate of 1 ml / min. dn/dc values in THF were determined using Optilab Rex detector.

Fourier Transform Infra-Red (FTIR) spectroscopy: FTIR spectra were recorded at room temperature on a JASCO 3600 FTIR spectrometer.

Dynamic Light Scattering (DLS): A Malvern Zetasizer Nano ZS apparatus was used to determine the hydrodynamic radius of the copolymers and the corresponding SCNPs at room temperature in THF solvent. The “size distribution by number” plot was employed in this work.

S4. Supporting Data

A. Determination of the degree of functionalization through ^{1}H NMR spectroscopy.

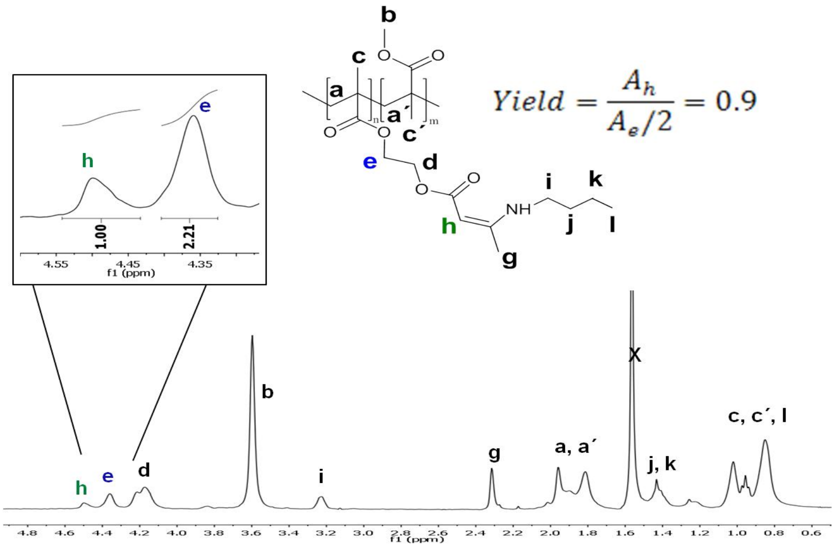


Fig. S1. Content of alkyl amine in the functionalizated copolymer as determined by ^{1}H RMN spectroscopy. Upon mixing copolymer and butylamine the ^{1}H NMR spectrum shows the characteristics bands of enamine formation at 3.2 ppm (**i**) and 4.5 ppm (**h**).

B. Comparison of hydrodynamic sizes by DLS measurements.

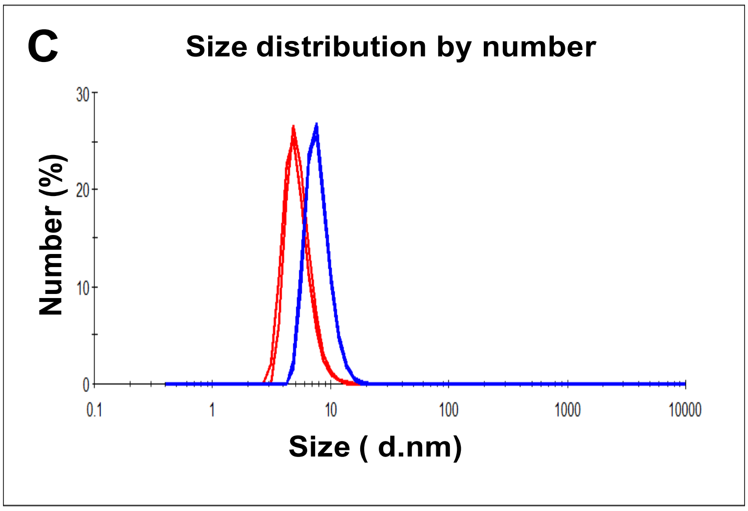


Fig. S2. Hydrodynamic diameter (D_h) distribution for **P1** (blue line) and **NP1** (red line), as determined by DLS. The average D_h changes from 7.8 nm to 5.3 nm upon SCNP formation.

C. Assembly / disassembly of SCNP NP1 as determined by FTIR spectroscopy.

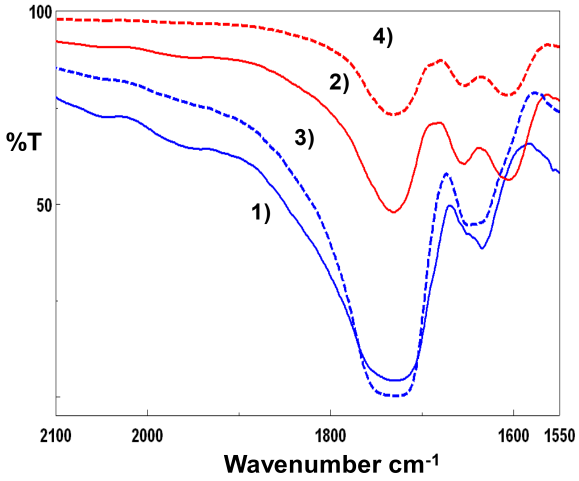


Fig. S3. FTIR spectra corresponding to: **1) P1** (blue line), **2) NP1** (red line), **3) P1´** (broken blue line), and **4) NP1´** (broken red line).

D. Illustration of the facile one-pot assembly / disassembly of SCNPs containing enamine DCBs as determined by SEC/MALLS.

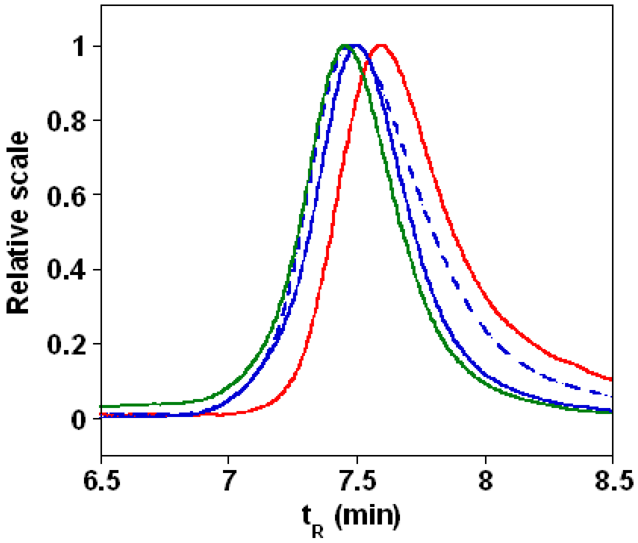


Fig. S4. Facile one pot SCNP assembly / disassembly, according to Scheme 1 (see main text), as demonstrated by SEC measurements: SEC trace of **P2** (continuous blue line), SEC trace of **FP2** synthesized directly from **P2** and **6** (hexylamine) (green line), SEC trace of SCNPs **NP2** synthesized through dynamic alkyl amine **6** / alkyl diamine **4** exchange (red line), and SEC trace of **NP2** after H_3PO_4 addition (**P2´**) (broken blue line).

E. SCNP disassembly of as a function of pH and reaction time.

Table S2. Disassembly degree as a function of pH and reaction time

pH	Reaction time (h)	Disassembly degree[a]
2-4	10	100 %
5	16	100 %
6	24	40 %

[a]Disassembly degree as determined from SEC retention times according to:

Dissasembly degree (%) = $(\mathbf{t_f} - \mathbf{t_R}) / (\mathbf{t_f} - \mathbf{t_0}) \times 100$

$\mathbf{t_0}$ = Retention time of the precursor.

$\mathbf{t_f}$ = Retention time of the SCNP.

$\mathbf{t_R}$ = Retention time of the SCNP after acidic treatment.

pH was estimated simply using paper by adding one drop of water to one drop of the reaction mixture and then adding this solution to pH paper. The pH of the reaction was controlled by using very limited quantities of phosphoric acid, and the pH of 6 was achieved using a limited volume of acetic acid.